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PARASITOLOGY

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A STUDY OF THREE NON-FURCOCERCOUS CERCARIAE OBTAINED FROM *BULLINUS CONTORTUS* IN THE SUDAN.

BY R. G. ARCHIBALD, C.M.G., D.S.O., M.D.,
Director, Wellcome Tropical Research Laboratories,

AND A. MARSHALL,
Superintendent, Bacteriological Section, Wellcome Tropical Research Laboratories, Khartoum.

(With 6 Text-figures.)

INTRODUCTION.

DURING the course of bilharzial investigations, the various types of cercariae herein described were found infesting sinistral molluscs obtained from different parts of the Sudan.

TRANSPORT AND STORAGE OF MOLLUSCS.

In a country like the Sudan with limited railway communications, the successful transport of molluscs from outlying districts presents difficulties, especially when several weeks elapse between their collection and arrival at the laboratory.

For the successful transport of the molluscs (*Physopsis*, *Bullinus*, or *Planorbis*) it is essential that (1) overcrowding should be avoided; each adult snail should be provided with not less than 10 c.c. of water, (2) the water should be changed every three to four days, (3) grass and leaves should be added as food supply, and (4) a free supply of air should be assured. If gourds serve as receptacles for the transport of molluscs, they should be old because new gourds contain a water-soluble substance which is lethal to molluscs.

In the laboratory, shallow enamelled dishes filled with river water serve admirably for the storage of molluscs provided (1) they are kept on a verandah where there is ample light and a free circulation of air, (2) the water is changed every four to five days, and (3) handling of molluscs is avoided as far as possible. Grass with roots attached, and leaves of vegetables, should be added to reinforce the food supply.

METHOD OF STUDY.

The cercariae herein described were fully developed having escaped from infested molluscs. When a consignment of molluscs arrived, the water containing them was examined for cercariae; if these were found, the molluscs

were placed separately in a little distilled water in order to ascertain which snails were infested, the cercariae derived from individual molluscs being studied. When sufficient cercariae had been obtained, the infested snails were placed in river water with the necessary food supply.

Although fresh or unfixed specimens of free cercariae were studied in most cases, certain details were worked out on fixed and stained specimens. The organs of the infested molluscs were examined as a routine procedure.

Technique.

The cercariae were examined either in pure normal saline solution or in saline containing a freshly added dye in different concentrations, whereby *intra vitam* staining was obtained. Further details of their anatomy may be shown by mounting them in blood serum derived from man or horse, as suggested by one of us (A. M.). This technique has proved invaluable and is carried out as follows: a drop of water or normal saline containing cercariae is pipetted on to a slide and a drop of serum is added and the whole mounted under a coverslip. In such a medium the cercariae lose their activity and the "clearing effect" of the serum permits of a better study of anatomical details; moreover such preparations favour the study of the exact position of flame cells which continue active for a long period.[1]

Intra vitam staining: freshly prepared neutral red in different concentrations in normal saline gave the most uniform results; the concentration required varied for different cercariae. A weak solution of basic fuchsin in normal saline also proved useful especially for flame cells. When serum was used as a substitute for saline the best staining was obtained with weak cresolic acid solution.

For fixation, prior to staining, various reagents were employed. The best results, perfect fixation with extension, followed the use of hot lacto-phenol solution (equal parts of lactic acid, carbolic acid, glycerin and water), a few drops of which, heated almost to boiling point in a test-tube, were added to a watch-glass containing living cercariae in water. For staining purposes it was only necessary to add a diluted dye to the lacto-phenol solution in the watch-glass, and allow the cercariae to stain for 12–24 hours. Overstaining may be reduced by adding a little more lacto-phenol. Permanent preparations were made by mounting the stained cercariae in equal parts of lacto-phenol and a gum solution. The latter was prepared as follows: gum tragacanth 3 parts, gum acacia 1 part, water 100 parts; dissolve by boiling, add equal parts of lacto-phenol and filter. The filtrate constituted an excellent mounting medium. Borax carmine (alcoholic solution) gave the best staining results with lacto-phenol fixed cercariae. Dilute Delafield's haematoxylin also gave good results.

The merits of lacto-phenol solution as a fixative of cercariae may be

[1] The choice of human or horse blood serum depends on the type of cercariae being studied The cercariae of human schistosomes appear to be adversely affected by horse serum, but can be studied with advantage in human serum.

summarised as follows: (1) applied as a hot solution it gives immediate, perfect, and permanent fixation with the cercariae fully extended; (2) a dye may be added to it for purposes of staining the cercariae over a prolonged period; (3) it prevents overstaining, and has also a clearing effect permitting a better study of anatomical details; (4) it allows of cercariae being easily removed with a pipette, no trouble being experienced through their adhering to glass; (5) it serves as a suitable mounting medium.

Measurements of cercariae were made according to the method recommended by Manson-Bahr and Fairley (1920). Free, actively motile cercariae in water were placed on a slide, which was then warmed over a spirit flame for 5–8 seconds to kill the cercariae, a coverslip was then gently applied and the cercariae measured. There should be enough water on the slide to prevent undue compression of the cercariae when the coverslip is applied.

NOMENCLATURE.

Miller (1926), in his excellent monograph, has rightly called attention to the lack of uniformity in the nomenclature employed by different workers in describing certain organs of cercariae. Consequently we have adopted a nomenclature in this paper which, in the main, is in accord with Miller's suggestions.

The glands found in the anterior organ or sucker have been designated cephalic or head glands, whereas the large unicellular glands posterior to that organ and designated by various workers as salivary, mucin, secretory, and periacetabular glands have been termed "penetration glands," a term originally suggested by Miller as indicating rather their function than their position in the body of the cercariae.

The post-larval development of our cercariae was not studied, consequently no attempt has been made to classify them in related groups wholly on the basis of larval characters. Numbers have been given to them in preference to names indicating specific peculiarities, a procedure adopted by Sewell (1922) in his studies on Indian cercariae.

Cercaria sudanensis No. 1.

(Figs. 1 and 5.)

Host: *Bullinus contortus*.

Locality and date: A *khor* (rain-water course) at Gebel Dud, 30–40 miles distant from the Blue Nile, Blue Nile Province. October, 1930.

The khor contained some vegetation and aquatic plants, and harboured aquatic birds. For six months in the year it provides the water supply for the inhabitants and stock of a few native villages; for the remainder of the year the khor is dry.

The *B. contortus* collected were full grown and half grown; at least 80 per cent. harboured this cercaria, but did not appear to have an adverse effect on the longevity of the molluscs even when kept under laboratory conditions.

This cercaria, when viewed *in vitro*, is most active, it swims with a rapid

vibratory movement curling the body and contracting it into a spherical mass, which is driven through the water by the vigorous lashing of a muscular tail; at times the movements slow down and the cercaria extends itself and adheres to the side of the glass tube or bottom of a watch-glass by means of a strong ventral sucker, adopting a crawling movement, body foremost, by alternate expansion and contraction. After several hours in a watch-glass containing water, their movements become more sluggish and the body assumes a triangular shape, the apex of the triangle pointing posteriorly. When viewed laterally the body presents a concavo-convex appearance with the anterior end concave ventrally. The body is capable of considerable extension and contraction more especially in its anterior two-thirds. The muscular tail stem, with powerful longitudinal and circular muscles, is ventrally fixed, fitting into a concavity at the posterior end of the body; it has a well-developed cuticle which is expanded on one side at a little more than its lower third to form a fin fold or membrane which, when extended as a fine membrane posteriorly for a short distance, appears on the opposite side of the tail stem. Within the membrane there are hyaline rods or rays which appear to act as supports. The tip of the tail is represented by a sharp conical hyaline process which is frequently curved at the end like a hook. This conical process is capable of contraction and extension, and shows a few rounded nuclei within, but is devoid of spines.

The body-length ranged from 380–420μ, while its maximum breadth ranged from 188–232μ. The tail, when extended, measured 560μ with a basal diameter of 48–60μ. There is a thick well-developed spinose cuticle raised in the form of annulations or ridges which surrounds the greater part of the body and the whole of the tail with the exception of the tip. On the body this cuticle appears to be limited anteriorly by the shoulder of the cercariae where a single row of thick straight spines can be seen arranged like a palisade. Normally this row of spines is posterior to the mouth; when the cercaria is moribund, the mouth is frequently retracted to a position posterior to this row of spines. In the centre and just posterior to this row of spines there is a curious collection of thick hyaline spikes, arranged in the form of a crown and pointed anteriorly with a broader base posteriorly.

The anterior end of the cercaria is bluntly rounded and is occupied by an anterior organ (48–60μ in diameter), which is more or less circular when the cercaria is at rest; within this organ is a sub-terminal mouth guarded by a row of spines.

The powerful ventral sucker or acetabulum is situated in the anterior part of the posterior half of the body, and is very prominent and somewhat funnel-shaped; it is guarded by a single row of thick spines.

The mouth opens into a short prepharynx, which leads into a well-developed pharynx and this, in turn, is continued into a long segmented oesophagus, which bifurcates near the acetabulum into two blind sacculated caeca that end posteriorly about the level of, and external to, the bladder.

No penetration glands were apparent, nor were eyespots detected either in fresh or stained preparations.

The excretal bladder situated in the posterior portion of the body, just anterior to the junction of the tail stem, is a powerful contractile organ consisting of a small posterior sac connected with a large middle sac with lateral horns, from which emerge one pair of posterior excretory tubes; the middle sac in turn communicating with a large anterior sac with lateral horns which receive a pair of anterior excretory tubes. The posterior sac is connected with the caudal excretory tube which extends down the tail to end laterally near the base of the conical spike. The posterior excretory tubes arising from the middle sac extend forward to the base of the anterior organ, then, forming a loop, become continuous with the tubes containing a series of spherical refractile excretal granules with lateral branches as figured in Fig. 1; the anterior excretory tubes, which are connected with the anterior sac of the bladder, extend forward, and, in the vicinity of the acetabulum, open into the tubes containing the refractile excretory granules. There is no communication between the anterior excretory tubules.

It may be mentioned that the study of the course of these excretory tubules was only rendered possible by mounting the cercariae in a medium containing serum. Fourteen pairs of flame cells were present in the posterior excretory tubes, and four pairs in the anterior excretory tubes, making a total of eighteen pairs of flame cells in the body. Flame cells are absent in the tail. Oval cells containing granules (cystogenous cells) are present scattered throughout the body; specially differentiated cells representing the genital organs could not be found.

The cercariae apparently develop from a sausage-shaped redia (Fig. 5), and also from cysts, both present in large numbers in the snail's liver. The rediae contain a golden yellow pigment, are motile, and show a well-developed pharynx opening into a rhabdocoel gut containing brownish black pigment granules; two or more immature cercariae are present within the rediae, the posterior portion of which is filled with germ cells. The cuticle of the redia is markedly annulated or ridged; at the anterior end is a well-developed lateral birth pore and more posteriorly a prominent locomotor appendage. The cysts are circular, show a double contoured wall and measure 192μ in diameter; in the interior of the cyst there can be seen the anterior portion of the body of the cercariae showing anterior organ, pharynx and the refractile granules of the excretory tubes; brownish black pigment particles are also observable. Encystation does not appear to occur outside the molluscan host.

Cercaria sudanensis No. 2.
(Figs. 2 and 4.)

Host: *Bullinus contortus.*

Locality and date: Gebel Dud, Blue Nile Province. October, 1930.

About 3 per cent. of the molluscs collected were infested with this cercaria; the infested molluscs were but a little more than half grown.

This is a small cercaria which exhibits active movements by means of a small tail. When examined in water *in vitro* this cercaria shows a preference for the upper layers of the fluid: swimming upwards, then ceasing movement, and slowly sinking downwards; under such conditions it shows considerable activity at the end of twelve hours. Viewed in profile the body is curved ventralwards producing a concavo-convex appearance, and is capable of considerable extension and contraction. In heat-killed specimens the average measurements of the body were $280\mu \times 110\mu$, and of the tail $174\mu \times 24\mu$ (basal breadth). The body in the semi-contracted state is somewhat pyriform, broader anteriorly; the tail shows a ventral attachment. At its site of attachment, the body cuticle is thickened, and there are a pair of lappets formed by the postero-ventral angles of the body, within which the caudal pocket shows a hyaline lamina containing three or more spines. The body and tail show marked annulations of the cuticle, the whole surface of which is spinose. On the dorsum there is a cuticular sheath covering the anterior two-thirds of the body. The anterior organ (60μ in diameter) has a centrally placed muscular mouth from which a spear-shaped stylet emerges; this stylet (20μ long, 6μ broad basally) is pointed anteriorly; on each side of the stylet, and within the anterior organ, there is a cephalic gland. There is a well-marked prepharynx opening into a muscular pharynx, which leads into a short oesophagus that bifurcates into two narrow and short caeca ending about the level of the middle of the acetabulum. The acetabulum, situated in the middle of the body, is a powerful organ 60μ in diameter with prominent muscular basal attachments. The opening is guarded by spines and varies in shape, being either circular or transversely oval according to the movements of the cercariae. There are eight pairs of pear-shaped penetration glands situated antero-laterally to the caeca; each gland shows a well-marked nucleus, and a fine granular cytoplasm with no definite affinities either for acid or basic dyes. Only two gland ducts can be traced, these extend forward and terminate in the anterior part of the anterior organ; it is difficult to ascertain the number of ducts owing to the cellular nature of the body, and its cystogenous cells.

The excretory system is represented by a muscular bladder consisting of two sacs connected by an isthmus; the larger posterior sac is continuous with the caudal excretory tube, which traverses the whole length of the tail and ends terminally. From the anterior sac originate two lateral cornua; these are connected with two very convoluted and thickened excretory tubules which can only be traced anteriorly as far as the middle of the body where their

further ramifications are lost among the numerous body cells. Two pairs of flame cells are noted in the posterior fourth of the body, one pair close to the excretory bladder, the other pair external to the anterior cornua; the excretory tubes connected with these flame cells could not be traced; the tail shows no flame cells. Eyespots are absent. Posterior to the acetabulum there are collections of granular cells which may represent the genitalia.

This cercaria is capable of living in water outside its host for a comparatively long time, and under certain conditions is capable of encystment. The cysts, 184μ in diameter, are sub-circular (see Fig. 4).

This cercaria develops in finger-like sporocysts in the snail's liver. These sporocysts are white, and contain second generation sporocysts.

Cercaria sudanensis No. 3.
(Figs. 3 and 6.)

Molluscan host: *Bullinus contortus*.

Locality and date: Khor Abu Habl, Kordofan. November, 1930.

This is a large cercaria infesting at least 5 per cent. of the molluscs. It is a fairly active swimmer with no particular proclivity for either the top or bottom layers of the medium. It swims by means of lashing movements of the tail with the body bent dorso-ventrally, sinking as soon as movement ceases and rising again when the tail becomes active. Viewed laterally the body is curved dorso-ventrally, and the tail has a ventral attachment. At the bottom of a watch-glass containing water the cercaria can be seen performing a crawling movement by attaching itself with the ventral sucker and extending its body and drawing up its tail to the position previously occupied by the body. In a semi-contracted state the body is pear-shaped with the apex anteriorly; it has considerable powers of extension and contraction. The muscular tail is often slightly curved at its blunt tip.

The whole cuticular surface of the body and tail stem is spinose; beneath the cuticle are very powerful longitudinal and circular muscle fibres. These, especially the circular fibres, appear to be absent in the extreme tip of the tail.

Just posterior to the anterior organ there is a slight shoulder which forms the anterior limit of attachment of the cuticular membrane.

The body in heat-killed specimens is about 300μ long and 140μ broad, the tail is 570μ long and 50μ broad basally.

The anterior organ is a slightly oval powerful muscular structure, 52μ long and 48μ broad, with a sub-terminal muscular sucker or mouth guarded by spines; at the posterior margin of the anterior organ are situated two cephalic glands. There is a short prepharynx leading into a strong muscular pharynx which, in turn, leads into a long oesophagus, the interior of which is granular. The oesophagus extends posteriorly, and bifurcates just anterior to the acetabulum into two narrow granular caeca which end in the vicinity of the excretory bladder.

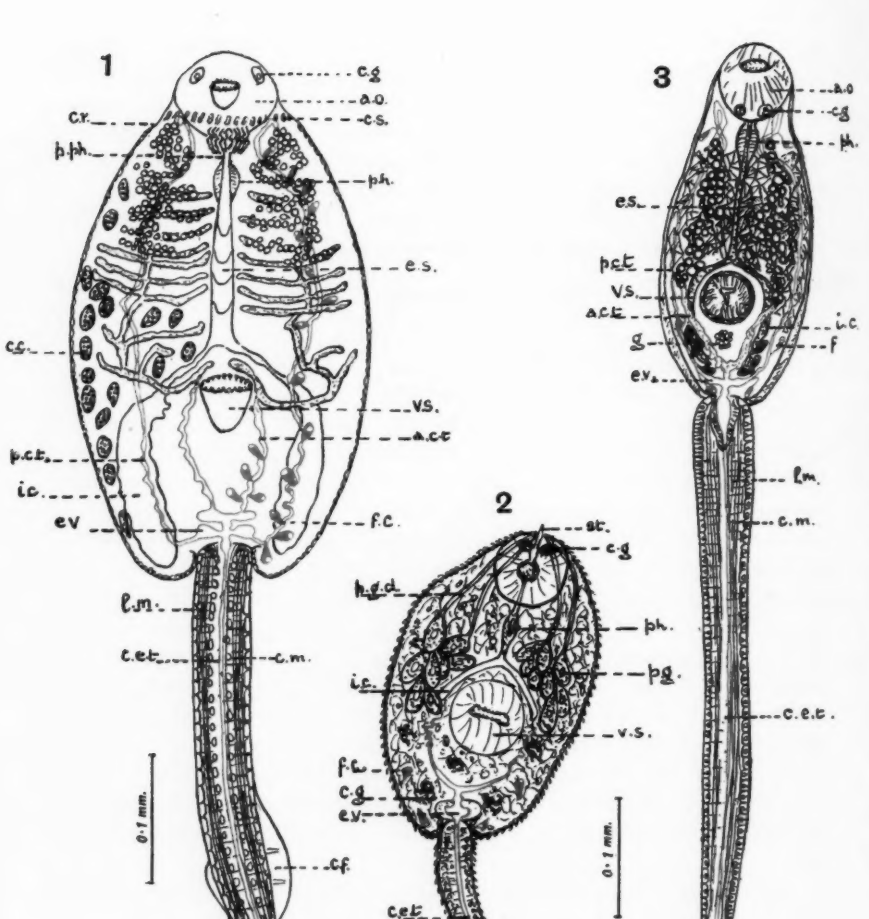

Fig. 1. *Cercaria sudanensis* No. 1 (see Text, p. 273).
Fig. 2. *Cercaria sudanensis* No. 2 (see Text, p. 276).
Fig. 3. *Cercaria sudanensis* No. 3 (see Text, p. 277).

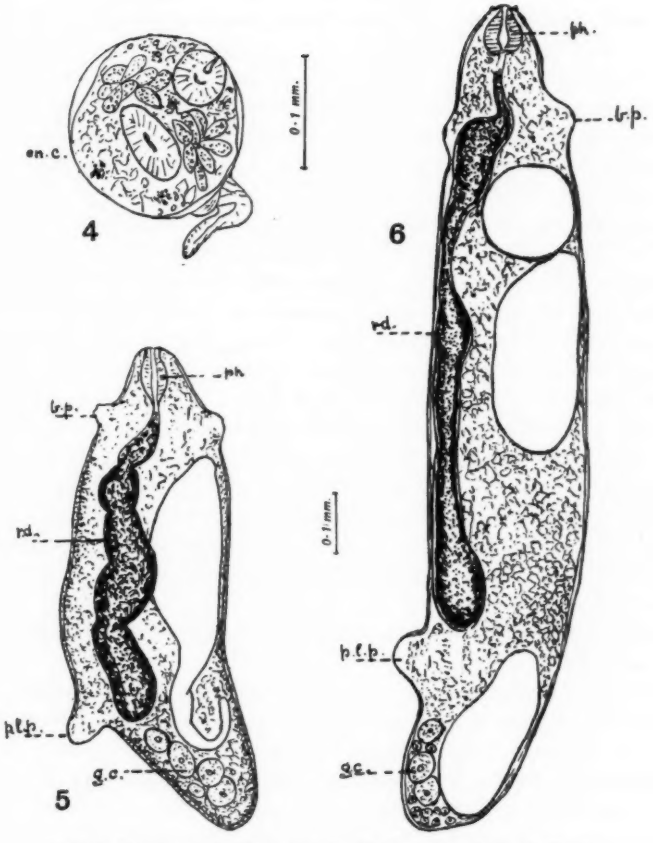

Fig. 4. Sporocyst showing cercariae of *Cercaria sudanensis* No. 2.
Fig. 5. Redia of *Cercaria* No. 1.
Fig. 6. Redia of *Cercaria* No. 3.

The powerful acetabulum, 52μ in diameter, lies in the anterior portion of the posterior half of the body; it has either a circular or Y-shaped opening depending on whether the body is at rest or extended. The acetabulum has a very muscular base 20μ thick. Posterior to the acetabulum, a collection of round cells represents the genitalia. The excretory bladder is tripartite, consisting of a posterior sac extending like a cone into the base of the tail stem, and is surrounded by bands of muscle fibres; this cone is continued posteriorly in the form of the caudal excretory tube, which terminates on the ventral surface of the tail stem in a small oval excretory pore situated about 50μ from the tip of the tail. The cone is connected anteriorly by an isthmus to the middle sac, from which two posterior horns extend laterally, and the middle sac is in turn connected by an isthmus to the anterior sac. The anterior excretory tubes open into the antero-lateral anterior margins of this sac; these tubes are somewhat sinuous and thick-walled and pursue an outward and forward course to about the level of the anterior margin of the acetabulum, and from this point forward to the level of the pharynx; they are filled with somewhat large refractile excretory granules. Opposite the pharynx each duct loops on itself and turns backwards. The marked cellular structure of the body renders it difficult to trace accurately the further course of this tube, but it apparently extends backward to the posterior part of the body as a fine duct which terminates in a flame cell near the excretory bladder. There are six pairs of flame cells in the body; one pair on a level with the pharynx and five pairs posterior to the acetabulum. There are no flame cells in the tail.

The greater part of the body presents a characteristic tessellated appearance due to the presence of closely packed rods containing lozenge-shaped cells whose character remains undetermined. Eyespots and salivary glands are absent.

Beneath the cuticle of the tail there are longitudinal and circular muscle fibres and nucleated cells.

This cercaria develops in motile rediae (Fig. 6) 1–1·5 mm. long and 0·25 mm. broad. The rediae are somewhat sausage-shaped. The narrow anterior end bears two lateral prominences corresponding to birth pores, and at the posterior end there is a locomotor process. The muscular pharynx leads into a rhabdocoel gut containing dark pigment. The remainder of the redia contains immature cercariae and golden-coloured pigment granules.

Summary.

1. A technique is given for the detailed study of living cercariae in which reference is made to the value of human or horse blood serum as a mounting medium.

2. As a fixative agent for cercariae prior to staining the use of hot lactophenol solution is recommended.

3. A description is given of three non-furcocercous cercariae obtained from *Bullinus contortus* in the Sudan.

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LETTERING TO TEXT-FIGURES.

act. anterior collecting tube; *ao.* anterior organ; *bp.* birth pore; *cc.* cystogenous cell; *cet.* caudal excretory tube; *cf.* caudal fin fold; *cg.* cephalic gland; *cm.* circular muscle; *cr.* collar region; *cs.* collar spines; *enc.* encysted cercaria; *ep.* excretory pore; *es.* oesophagus; *ev.* excretory vesicle; *fc.* flame cell; *g.* genitalia; *gc.* germ cell; *ic.* intestinal caecum; *lm.* longitudinal muscles; *pct.* posterior collecting tube; *pg.* penetration gland; *pgd.* penetration gland duct; *ph.* pharynx; *plp.* posterior locomotor process; *pph.* prepharynx; *rd.* rhabdocoel gut; *st.* stylet; *vs.* acetabulum.

Note: The figures represent camera lucida drawings and the excretory system is figured red.

(*MS. received for publication* 11. II. 1931.—Ed.)

SARCOSPORIDIOSIS IN DUCKS.[1]

By WILLIAM A. RILEY.

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(With Plate XI.)

That ducks are subject to infestation by sarcosporidia has long been known. It seems to have been emphasised particularly because ducks afforded one of the first-known instances of the occurrence of these puzzling protozoa in birds. Very few new records have been published until now and no adequate illustrations of the gross features of the infestation have appeared. A general review of the subject and presentation of additional cases, with illustrations, therefore seems desirable.

The first mention of "psorosperms" in ducks dates back to Joseph Leidy, the well-known discoverer of trichinae in pork, etc. In a report to the Philadelphia Academy of Natural Science, dated April, 1875, he records the occurrence of "psorosperms" in a mallard which had been submitted to him by the ornithologist, Elliot Coues. In the interstices of the muscle were "oval, white bodies from one to two-tenths of an inch long and about one-third of a line thick," filled with "myriads of fusiform corpuscles, resembling minute naviculae and measuring about 1–1500th of an inch in length." Leidy calls attention to the resemblance of these bodies to those discovered by Müller in many fish and described by him under the name of psorosperms. Concerning the method of infection of the duck he makes the somewhat contradictory statement that "although the Mallard is not a fish eater, the bird may have become infected by having swallowed an infected fish."

It is worth recording that C. V. Riley (1869), when State Entomologist of Missouri, examined a wild mallard "which was so full of a new and undescribed cystadid, that you could not put your finger upon any portion of the flesh without touching some." He regarded them as larval cestodes, comparable to those of *Taenia solium* in pork and states that "if introduced along with a portion of the raw flesh of the duck, into the human stomach, they would probably have developed into some kind or other of intestinal worm." This misinterpretation of Riley led Neveu-Lemaire (1912) to speak of *Sarcocystis* as causing in the United States a disease known as *ladrerie* (measles) of the duck. The term has never become established in the literature of this country.

It remained for Stiles (1893) to recognise these bodies as sarcosporidia and to describe as new the species found in the duck. He had before him

[1] Published as Paper No. 983 of the *Journal Series of the Minnesota Agr. Exp. Station.*

material from two shovel-bill ducks (*Spatula clypeata*), one undetermined duck, and one mallard (*Anas platyrhyncha*)—apparently the material submitted by Coues to Leidy. The parasite he named *Balbiania rileyi* in honour of C. V. Riley who was at that time Government Entomologist. The generic name *Balbiania* was used because of the inter-muscular position of the cysts, but it is now understood that this distinction is not generic and the species is known as *Sarcocystis rileyi* (Stiles, 1893).

Subsequent to the work of Stiles there have been very few original references to the occurrence of this sarcosporidian. Crawley (1911) studied its microscopic structure. Hall (1925) observed heavy infestation in a domesticated duck purchased in the market at Washington, D.C., and suggests the possibility that the parasite might cause economic loss at times in some localities. Von Betegh and Dorcich (1912) state that Betegh in the course of thirteen years found three cases of generalised sarcosporidiosis of domesticated ducks. It does not appear that other references in the literature to the occurrence of *S. rileyi* in domesticated ducks are based on original observations.

The writer's attention was first directed to this parasite in 1919 when there was submitted to him a specimen of a wild mallard duck shot at an island in the Mississippi River, just below St Paul, Minnesota. Although it was shot on the wing, in apparently normal flight, a glance at the accompanying illustration (Pl. XI, Fig. 1) explains why it was looked upon with suspicion when the hunter dressed it for roasting.

All of the skeletal muscles were infested to an extreme extent. The flesh was literally packed with the creamy white cysts, measuring from 4 mm. to 8 mm. in length and about 2 mm. in diameter. It was estimated that some 2000 cysts were present in an ounce of the meat.

At about this same time there was described to the writer a condition in a shovel-bill duck from Minnesota, which was apparently due to a similar infection.

Although such unusual material is very likely to be submitted to the University, or to the State Game and Fish Department, with which we have been in close touch, no other cases came to our attention until this fall (1930), when, within a week's time, two additional specimens of infested Minnesota ducks were submitted by Commissioner McCullough of the State Game and Fish Department. The first of these, a wild mallard drake, proved to be infested to the same extreme degree as that noted in the original case. Pl. XI, Fig. 2, shows the cysts in natural size as they lie in the interfascicular connective tissue of the breast muscle, while Fig. 3 shows their appearance in cross-section.

Cases of sarcosporidiosis in ducks are so rare and the conditions under which the birds are killed such that any symptoms of disease might readily be overlooked. In the literature dealing with mammalian infestations are numerous references to alleged paralysis, chiefly of the hind quarters, feebleness and other symptoms. McGowan (1923) regards the parasites as the cause of a serious disease of sheep which manifests itself under two varieties—the

"itchy" and the "paretic." At autopsy he found the skeletal muscles to present an extremely watery, gelatinous appearance, and, on microscopic examination, found them heavily laden with sarcocysts.

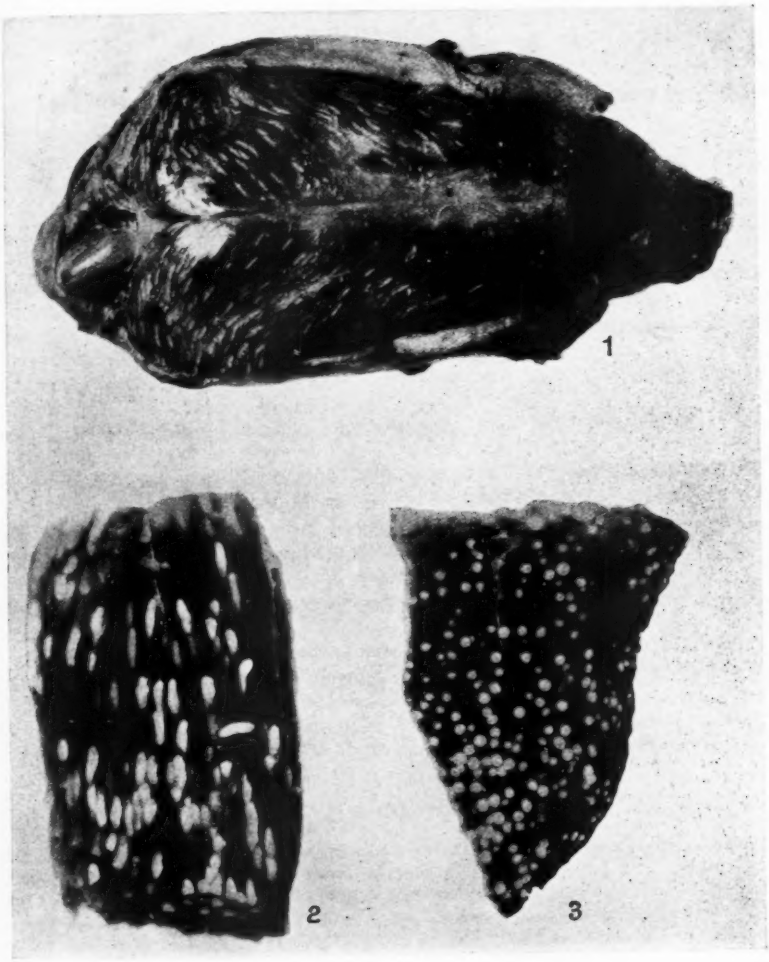
No indication of any such effects were seen and neither are any noted in the scant literature dealing with the sarcosporidia of birds. However, the parasites at the outset develop within the muscle fibres, and such heavy infestations must represent an enormous destruction of muscle tissue. Apart from this, it is difficult to understand how such heavily laden muscle is able to function. And yet, in none of the cases were there noted any evidences of abnormal flight. The condition was not suspected before the birds were dressed for cooking.

Concerning possible sources of infection of these birds nothing is known and little can be known until there is more understanding of the life-history of the sarcosporidia in general. All of the experimental evidence available at present points to the conclusion that the infection of new hosts occurs through eating of the ripe cysts in flesh, or through food which has been contaminated by the excrement of infested animals. The first-mentioned method, revealed by the work of Theobald Smith (1901), has been verified so often that, in some laboratories, it affords a routine procedure for obtaining material for study. Negri (1907) found that faeces of mice infected by eating muscle containing *S. muris* are infective for 15 to 60 days thereafter. This has been confirmed repeatedly by subsequent investigators, and Scott (1920, 1930) has presented clear evidence of a similar possibility in the case of *S. tenella*.

Bearing on the problem is also a growing mass of evidence indicating that the sarcosporidia possess a low degree of host specificity. It is probable that not many workers would accept the extreme view of Alexeieff (1913), who considers that all of the described species should be classed under the one name *Sarcocystis miescheriana*. Nevertheless it is very significant that *S. muris* and *S. tenella* may develop in a wide range of hosts and that a striking difference in the gross morphology of the parasites in different hosts is observable.

Regarding the possible mode of infestation in ducks, the most significant experimental work is that of Betegh and Dorcich (1912) who succeeded in infecting a chicken and two domesticated ducks by feeding them with *S. tenella* from sheep. As in all experiments of this nature the sarcosporidia developed slowly. The chicken was killed 3 months after the first feeding, the ducks after 5½ months. The macroscopic examinations were negative, but microscopic sections of muscle from all three birds showed a generalised sarcosporidiosis.

The food of wild mallard ducks is varied, but McAtee (1918) has shown that it is nine-tenths vegetable. They not only feed on leaves and stems of marsh plants, which may be contaminated by faecal material from cattle or sheep as well as from infected ducks, but they have been found feeding in large numbers in cornfields and in wheat and barley stubble where there is still more opportunity for picking up infective material. This must be kept in mind in considering possible sources of infestation.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Regarding the edibility of the birdflesh in the condition illustrated in Pl. XI, there is little to say. Hall (1925) comments: "on esthetic grounds, if for no other reason, the flesh of the duck in question (domesticated duck reported) would be adjudged as unfit to eat." The question of edibility was raised regarding each of the cases in wild ducks reported by me in this paper. Apart from the esthetic grounds and the generally accepted view that heavily parasitised flesh is unwholesome, the repeated demonstrations of toxic substances derived from sarcosporidia strongly suggest that there may be danger in the consumption of such excessively infested birds.

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DESCRIPTION OF PLATE XI.

Fig. 1 (reduced). Breast of mallard duck, *Anas platyrhyncha* from Minnesota, showing generalised infestation by *Sarcocystis rileyi* (Stiles, 1893).

Fig. 2 (natural size). Cysts of *S. rileyi* in breast muscle of mallard duck.

Fig. 3 (natural size). Cross sections of cysts of *S. rileyi* in breast muscle of mallard duck.

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A DESCRIPTION OF *EMBADOMONAS* n.spp. FROM *BLATTA ORIENTALIS, RANA TEMPORARIA, BUFO VULGARIS, SALAMANDRA MACULOSA*; WITH A NOTE UPON THE "CYST" OF *TRICHOMONAS BATRACHORUM*.

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(With Plate XII.)

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Whilst attempting to cultivate the intestinal protozoa of frogs and toads I obtained growths of a flagellate belonging to the genus *Embadomonas* Mackinnon. Finding no detailed description of this flagellate, and as, with the exception of the Embadomonads from the larvae of *Tipula* and *Trichoptera* described by Mackinnon (1915), and of *Embadomonas cuniculi* described by Collier and Boeck (1926), little seemed to be known of the process of division in these flagellates, I determined to isolate it from the other protozoa present in the primary cultures in order to study its morphology, method of division and life-history *in vitro*. Some months later I was given a *Salamandra maculosa* whose intestinal protozoa I attempted to cultivate and from which I obtained a strain of *Embadomonas* free from other protozoa. During the last few months I have become interested in the small flagellates living in the hind gut of *Blatta orientalis*, in smears of the gut contents of which I have found a species of *Embadomonas* to my knowledge not previously described.

On several occasions I have had the opportunity of discussing my findings with Mr Clifford Dobell, F.R.S., who has given me kindly criticism and advice and has read through my completed manuscript. I should like to offer to him,

here, my thanks for the interest he has shown and for the trouble he has taken. I am also indebted to Prof. J. Stanley Gardiner, F.R.S., Zoological Department, Cambridge, for kindly providing me with frogs, toads, cockroaches, and a salamander.

I. Material and methods.

Intestinal contents for the inoculation of cultures were obtained from the frogs and toads by the method described in a previous paper (Bishop 1931), wherein will be found descriptions of the culture media employed in the following study, and the methods used in the isolation of strains of protozoa from incompatible organisms, together with references to the relevant literature.

One strain of *Embadomonas* from an English frog, *Rana temporaria* L., was cultivated on coagulated whole-egg slopes partly covered with horse-serum diluted 1 : 8 in Ringer's fluid. Sterile rice-starch was added to the earlier sub-cultures until *Blastocystis* was exterminated. *Embadomonas*, unlike *Trichomonas* (Bishop 1931), never ingests grains of rice-starch since, though the cytostome is large, the actual orifice into the endoplasm is small. This strain of *Embadomonas* was cultivated for over five months, and merely abandoned, in a flourishing condition, because it was of no further experimental interest to me.

A strain of *Embadomonas* from an English toad, *Bufo vulgaris* L., was cultivated upon inspissated horse-serum slopes partly covered with horse-serum diluted (1 : 10) in 0·5 per cent. NaCl. The *Embadomonas* from the salamander *S. maculosa* was cultivated by the same method.

So far my efforts to cultivate the *Embadomonas* from the cockroach have been unsuccessful.

The smears, either from cultures, or, in the case of the cockroach, of gut-contents mixed with dilute serum, were fixed in either Bouin's or Schaudinn's fixative. Aqueous iron haematoxylin, using both the mordant and the stain for twenty-four hours in turn, was the stain used throughout the study.

II. Morphology of *Embadomonas blattae* n.sp. from *Blatta orientalis*.

The genus *Embadomonas* was founded by Mackinnon (1911) for a small flagellate in the larvae of *Trichoptera*. She at first described it as possessing one flagellum. Later, however (1915), she described two species, *E. agilis* from the intestine of larvae of *Trichoptera* and *Tipula*, and *E. alexeieffi* from the intestine of the larvae of *Tipula*. In this later account she described a second flagellum and diagnosed the genus as possessing, amongst other characteristics of taxonomic value, two flagella "one acting as an organ of locomotion, and the other lying in the cytostome."

I have been unable to discover any description of the *Embadomonas* which I have found in the hind gut of cockroaches. As its cyst differs from that of

E. agilis (to which flagellate the *Embadomonas* from the cockroach is somewhat similar in form), I propose to call this new species *E. blattae.*

Embadomonas blattae n.sp. (Plate XII, figs. 1–6.)

E. blattae appeared in about 40 per cent. of the cockroaches I examined from a certain locality in Cambridge, but the infection, in some hosts, was very slight. I had made smears from several London cockroaches, obtained from one locality, whilst working at the National Institute for Medical Research, London, and some of these smears showed *Embadomonas.*

E. blattae measure, when fixed and stained, 6–9μ × 5–7μ, the majority being about 7μ × 6μ. As in *E. agilis* (Mackinnon 1915) the rounded anterior end of the flagellate is flexed, at some point in the anterior half of the body length, at an angle to the remainder of the body. The body of *E. blattae* is broader than that of *E. agilis* and the posterior end may be pointed (Plate XII, fig. 1) or rounded (fig. 2). There is, as Mackinnon described in *E. agilis* and *E. alexeieffi,* an enormous cytostome bordered by siderophilous wavy lips. The lip on one side of the mouth is less well developed than on the other. The body is covered by a definite periplast and is devoid of amoeboid movement. The spherical nucleus lies at the anterior end of the body and varies slightly in appearance in different individuals. A layer of chromatin immediately inside the nuclear membrane is constantly present. This, in optical section, appears like a ring. In some individuals the distribution of chromatin round the periphery is regular (fig. 2), but in others (fig. 1) a larger amount of chromatin is seen at one pole. The karyosome, central in position, may be a single small granule (fig. 1), or may consist of several granules grouped to form an irregular mass (fig. 2). The two flagella arise close together from a pair of minute basal granules situated on the edge of the nuclear membrane. These basal granules are so tiny that it is exceedingly difficult to distinguish them and in many individuals it is impossible to do so. One flagellum lies in the cytostome, the tip sometimes projecting slightly from the posterior end of the depression. This flagellum is very slender and may be very difficult, or impossible, to distinguish. The second, free flagellum, used in locomotion, is much thicker than the cytostomial flagellum. It is as long as or longer than the body.

The endoplasm may contain many bacteria in process of digestion.

Efforts to cultivate *E. blattae* and other small flagellates from the hind gut of the cockroach are still being made. The cockroaches I have examined have never been very heavily infected with *E. blattae.* I have not attempted to search for dividing forms, hoping to obtain these with less difficulty if my cultivation experiments succeed.

Cysts and precystic individuals of *E. blattae* are found in cockroaches infected with the flagellate. Almost spherical precystic forms, measuring 5μ or 6μ, are found. In these (fig. 3) the anterior flagellum is still free and no cyst wall has as yet been formed. The borders of the cytostome are plainly

seen, but appear less wavy than in the free flagellate, the cytostomial flagellum lies entirely within the cytostome, and the two tiny basal granules are visible in favourable preparations. In the few precystic forms which I have found the structure of the nucleus varies as in the free flagellate. In the example drawn no karyosome was visible. Food inclusions are few or absent.

The mature cyst is slightly pear-shaped (figs. 5 and 6), but if seen from the end (fig. 4) it appears round. Cysts measure 5–6μ × 4–5μ. A cyst wall can be distinguished, but I have never seen the pellicle of the free flagellate remaining round the cyst as described by Mackinnon for *E. alexeieffi*. The cysts show the same variety of nuclear structure as found in the free flagellates. In the cysts of the two species she studied Mackinnon (1915) described a disintegration of the nuclear membrane and the escape of chromatin in granules. This I have never seen in *E. blattae*. The basal granules can sometimes be detected. The borders of the cytostome persist, one side frequently appearing thicker than the other. In certain positions of the cysts (fig. 4) the borders of the cytostome appear to be twisted. In some cysts the fine cytostomial flagellum can be seen. The anterior flagellum becomes wound round the cyst immediately below the wall, and appears as a siderophilous fibre. This fibre sometimes crosses the edges of the cytostome and thus complicates the appearance.

I have seen nothing to suggest that division occurs within the cysts. All the cysts I have studied have been uninucleate.

III. Morphology and division of *Embadomonas dobelli* n.sp. from *Rana temporaria*, *Bufo vulgaris*, and *Salamandra maculosa*.

(Plate XII, figs. 7–22.)

An *Embadomonas* from the gut of the English frog was first mentioned and cultivated by Wenyon (1926). I have found *Embadomonas* and its cysts in the gut of both frogs and toads in England and obtained cultures from them (p. 287). The free flagellate appears in very small numbers in very few of the animals examined. Only one of the twenty toads examined showed it upon direct microscopic examination of the intestinal contents. But although it might be present only in small numbers in the intestine it multiplied enormously in the primary culture. Moreover, it appeared in the primary cultures made from several frogs which had been negative for it when the intestinal contents had been examined microscopically. Cysts were more numerous than free flagellates in animals infected with *Embadomonas*.

The average size of this species of *Embadomonas* lies between 6–11μ × 4–8μ, but occasionally I have encountered "giants" of 14μ. The other known species of *Embadomonas* vary considerably in size (see Table I).

In the *Embadomonas* from frog and toad the greatest breadth lies immediately behind the nucleus and the body tapers in a posterior direction to a point. The anterior end is rounded. The spherical nucleus is enclosed in a definite nuclear membrane, lies at the extreme anterior end of the body and appears in optical section (fig. 7) as a darkly staining ring of chromatin. In

individuals not preparing for division or encystation a karyosome is not usually present. In some individuals (fig. 8) irregular granules of chromatin can be seen, but, though the number of flagella had not increased, the large size of the body led me to infer that division was imminent and that the presence of the granules was due to this. Wenyon and O'Connor (1917) and Dobell and O'Connor (1921) describe and figure a central karyosome in the majority of individuals of *E. intestinalis* and Jepps (1923) also figures this structure. Faust (1923) figures a central karyosome and points out that the nucleus of *E. sinensis* is smaller in proportion to the size of the body than in *E. intestinalis*. Collier and Boeck (1926) describe a "central rounded ovoid karyosome" which is connected to a centrosome on the nuclear membrane by a rhizoplast. The centrosome is connected by rhizoplasts to the blepharoplasts. Mackinnon (1915) has described a central group of chromatin granules in *E. agilis* and *E. alexeieffi*.

In the *Embadomonas* from the frog and toad there is a large cytostome approximately half the length (or rather more) of the body. It is deepest at its anterior end, lies on the ventral side of the nucleus and is overhung at its extreme anterior end by a projecting hood of protoplasm. Its lips are well defined, and slightly undulating. In some preparations (fig. 8) it will be seen that the supporting fibril and lip extend round the anterior end of the depression and to its posterior end on one side. The posterior part of the other side appears to be devoid of a siderophilous fibril. (Individuals fixed in this position are few—the animal tending to turn over on to its "side" as in fig. 7.)

Food is ingested at a point a little lower than the posterior edge of the nucleus. Except when bacteria are actually being taken in I have been unable to detect the orifice. A rapid current is created down the cytostome by the cytostomial flagellum. There is a continual circulation of food-vacuoles in the body.

Unlike that of *E. blattae* the long anterior flagellum is very slender and delicate. It is almost as long as or a little longer than the body. Where in *E. blattae* the cytostomial flagellum is exceedingly delicate, in the *Embadomonas* from frogs and toads it is thick at its root and gradually tapers (figs. 7 and 8). It lies in the cytostome and projects some distance beyond its edge. It beats, in the living flagellate, in an undulating manner, and when fixed almost invariably has a sinuous appearance as if it were attached to the base of the cytostome by an undulating membrane. That this is not so I am convinced by observations on the living animal, for I have occasionally seen the flagellum flicked out quite straight. It is very seldom fixed in this extended state.

The two flagella arise from a pair of minute but separate blepharoplasts. They lie close to the inner side of the lip at the anterior end of the cytostome (figs. 7 and 8). Viewed from some angles (figs. 9 and 10) the anterior end of the lip crosses the rim of the nucleus and the blepharoplasts have the appearance of being set in the peripheral chromatin of the nucleus. The basal granules of this species, though small, are much easier to distinguish than in *E. blattae*.

The body is enclosed in a delicate pellicle, and though it shows great

pliability in squeezing between obstacles amoeboid movement never occurs. The body of this species is not characterised by the marked torsion of *E. blattae* and *E. agilis* (Mackinnon 1915).

The structure of the free flagellate of the strain of *Embadomonas* (fig. 31) cultivated from *Salamandra maculosa* was similar to that of the *Embadomonas* from frogs and toads.

I have found no difference in the process of division in *Embadomonas* from both frogs and toads. I have studied it in the living animal and also in fixed and stained preparations. From cultures of five to ten days old I obtained the largest number of dividing forms. The first sign of approaching division in the living animal is the appearance of two new flagella. How these are formed I am unable to say as I never discovered animals in the act of producing them, but from the fact that they are frequently of unequal length I conclude that they are outgrowths (figs. 10 and 11). They arise at the same point as the anterior and peristomial flagella and like the former are delicate and directed forward. There is no differentiation in these new flagella, at this stage, into thick and thin flagellum. This stage seems to be of long duration for I have watched living individuals for over thirty minutes before any further change has occurred. In stained preparations the blepharoplasts are seen grouped together (figs. 9, 10, 11). They are very difficult to distinguish as they are less separated than in the resting stage but the group seems to be formed (fig. 11) of four granules.

A number of irregularly scattered granules make their appearance in the nucleus and these become larger and more clearly defined (fig. 10). Very frequently they are difficult or impossible to count as they lie partly over one another, but their number seems to be regular, and, so far as I could ascertain, there appeared to be five or six of them. In some individuals the chromatin granules become massed in the centre of the nucleus (fig. 9). The peripheral chromatin immediately below the nuclear membrane still persists, but in many individuals the matrix of the central part of the nucleus stains more darkly and there is less contrast between the periphery and the centre.

In the living animal two of the anterior flagella separate from the remaining pair and move quickly, beating all the time, to the opposite pole of the nucleus. The cytostome becomes less marked. I have very rarely found individuals at this stage in my preparations. In the specimen shown in fig. 13 the cytostome had vanished and the chromatin from the peripheral ring was not very distinct. The centrodesmose joining the two groups of flagella is shown clearly. An individual showing the flagella and their basal granules arranged at opposite poles of the nucleus is drawn in fig. 12. The peripheral chromatin is shown and an irregular group of chromatin granules in the centre of the nucleus. The centrodesmose is not visible.

In the living animal the body next becomes rounded and a stage persists for some minutes in which the pairs of flagella can be seen beating a considerable distance apart. The nucleus is at first spherical, but gradually becomes

oval. The cytostomial flagellum seems to lose its distinctiveness, is quite straight and not easy to distinguish from the other flagella. The chromatin from the periphery becomes dispersed throughout the nucleus which stains very darkly. Distinct granules can however be seen embedded in it. I have been unable to distinguish anything approaching a normal equatorial plate. That the granules do undergo some kind of division is certain because in early stages (fig. 10), of which I have studied many examples, five or six granules are always present, whilst in the telophase (fig. 16) the same number can be seen in each daughter nucleus. In most intermediate stages (fig. 14) individuals appear to have a double number of granules as if they had divided. Two basal granules, one at the base of each flagellum, can be seen at either pole of the nucleus, and between one individual of each pair is a definite centrodesmose. The cytostome disappears completely. Mackinnon (1915) found that the chromatin in *E. alexeieffi* was present in the spindle in definite granules but she was unable to find an equatorial plate. She does not figure such a definite centrodesmose as I have found in *Embadomonas* from frogs and toads.

In the living flagellate the nucleus elongates rapidly and appears to be stretched out by the action of the beating flagella. Preparations of this stage were few and showed (fig. 15) an indefinite anaphase, the centrodesmose persisting between the basal granules, and the chromatin arranged in indistinct granules towards the poles.

In the living animal the elongated nucleus can be observed to constrict across its equator. In stained preparations five or six granules can be seen towards the poles (fig. 16). The basal granules are usually distinct and the centrodesmose appears more massive at this stage than at any other. That it joins one granule of each pair is quite distinct. In *Embadomonas*, therefore, unlike *Trichomonas* (Bishop 1931), there is no separation of the centrosome from the blepharoplast. One granule of each pair in *Embadomonas* is a true basal granule, associated only with its flagellum, whereas the other, in addition to this function, also performs that of the centrosome, and is therefore a blepharoplast.

According to Collier and Boeck's account (1926) of division in *E. cuniculi*, a spireme, which later gives rise to two bivalent chromosomes, is formed by the elongation of the karyosome. An intranuclear spindle is formed with a centrosome at either end and with a paradesmose connecting them. They did not determine the origin of the new flagella and basal granules. In their figures (figs. 6 and 7) only the original pair of flagella is shown though the intranuclear spindle and chromosomes are already formed. In fig. 8 however, in which the nuclear changes are slightly more advanced, a new cytostome and the two new flagella are already completed. The origin of the new basal granules, of the new pair of flagella, and the division of the centrosome is obscure, and their drawings are very indistinct. According to their account the old cytostome persists throughout division and is not absorbed as I have found in *Embadomonas* from frogs and toads. Mackinnon (1915) found that

the cytostome usually vanished during division in *E. alexeieffi*; but in a few individuals (fig. 11) the original one appeared to widen out and be divided between the two daughters.

In the living animal the body elongates rapidly (fig. 17) after the constriction of the nucleus. I have been unable to follow the stages in the deposition of the chromatin in the periphery. The centrodesmose gradually disappears and individuals show two faint lines through the cytoplasm, one of which is probably the vestige of the constricted nucleus and the other the remains of the centrodesmose. The body constricts in the centre (fig. 18) and the two daughters move apart. During the late telophase, or just before the daughters move apart, a differentiation between the two flagella at either end becomes marked and the cytostomes begin to form. The daughter flagellates immediately after division are much broader in proportion to their length than the normal individual. They are often almost leaf-like (fig. 19). For some time after division has occurred they trail behind them a long thin "tail," which is the remains of the centrodesmose and the last vestige of the connecting link of protoplasm. This gradually shrinks. In some individuals the remains of the centrodesmose is not totally absorbed for a long time and it gives the false impression of a very faint "axostyle."

Collier and Boeck do not figure or describe a long constriction and elongation of the body in *E. cuniculi* such as I have found in *Embadomonas* from frogs and toads. They portray (figs. 9 and 10) the daughter nuclei as constricting close together. Mackinnon (1915) however figures (fig. 15) an elongation of the body similar to that which I have found in my flagellates.

In cultures of all ages, in one strain which had been running for over three months and in another which had only been isolated two weeks, I obtained a large proportion of individuals in which the organs had been duplicated (fig. 20). These individuals had two cytostomes, one on either side of the body, two pairs of normally differentiated flagella, two pairs of basal granules, and two nuclei lying side by side at the anterior end. In the majority of cases the nuclei were of equal size. Such individuals were observed by Mackinnon (1915, fig. 2). She suggested that they might be "freaks" but added that "the possibility of a sexual process should be borne in mind." Wenyon and O'Connor (1917) saw individuals with double sets of organs in the species from man. They considered them to be dividing forms. Da Fonseca (1920) published a description in the *Brazil Medico*, 1917, of an organism which he called *Waskia wenyoni* and which he found in the intestine of *Cebus caraya*. The organism was the "double" form of an *Embadomonas*. He apparently found no normal, single individuals. Jepps (1923, fig. 6) shows an individual of *E. intestinalis* with two nuclei lying side by side, which she interprets as a stage in division. Collier and Boeck (1926, figs. 10 and 11) show individuals of *E. cuniculi* in which two nuclei, two sets of flagella, and two cytostomes lie side by side at the anterior end. These they regard as the end phase of division prior to cytoplasmic fission.

I have studied "double" individuals in preparations of living flagellates for long periods, but without observing any division of the body. Their behaviour appeared normal and they fed voraciously by both cytostomes! The majority were not larger than normal individuals. I was unable to detect any change in the nucleus, such as would occur if the condition was a stage in a sexual process. They appeared in every way similar to the "double" individuals which I studied in *Trichomonas hominis* (1931). I searched my preparations diligently hoping to discover how the daughter nuclei arrived at the position shown in "double" individuals, but without success. I found a number of individuals in which one nucleus (fig. 21) was almost twice the size of the other. This larger nucleus almost invariably contained scattered chromatin granules, as if preparing to divide. Very rarely I found individuals with three nuclei all of the same size, three cytostomes and three sets of flagella and basal granules (fig. 22). Such trinucleate individuals are so rare that it is impossible to consider that all double individuals will undergo further division of the nucleus. I have kept cultures containing many double individuals under daily observation, but without finding any further development or observing a division of such double organisms. This "double" condition, judging from the literature, occurs in the intestine as well as in cultures. Its significance is problematic.

Broughton-Alcock and Thomson (1922) studying *Embadomonas intestinalis* from human faeces figure (fig. 45) an individual with two nuclei, two cytostomes and two sets of flagella at the anterior end and regard this as a stage in binary fission. They also describe and figure individuals with four and five nuclei, but the relationship of the flagella and cytostomes is not shown.

I never observed "double individuals" in *Embadomonas blattae*, nor in the strain of *Embadomonas* from *Salamandra maculosa*.

The process of division in the *Embadomonas* from *S. maculosa* seems similar in every way to that in *Embadomonas* from frogs and toads.

IV. Encystation of *Embadomonas dobelli* n.sp. from *Rana temporaria*, *Bufo vulgaris*, and *Salamandra maculosa*.

(Plate XII, figs. 23–29.)

Mackinnon (1915) first described the cysts of *Embadomonas alexeieffi* and *E. agilis*, discovering them when she first found the flagellate. Wenyon and O'Connor (1917) found the cysts of *Waskia* [= *Embadomonas*] *intestinalis* in faeces. They give the measurement as 4·5–6 μ in length—a measurement corroborated by Dobell and O'Connor (1921). Hogue (1921) obtained cysts of *Waskia* [= *Embadomonas*] *intestinalis* in culture in large numbers. She observed that they were pear-shaped and measured 4·8–7·2 $\mu \times$ 4–4·8 μ. Collier and Boeck (1926) found cysts of *E. cuniculi* in their cultures as well as in the rabbit. These cysts measured 5–6·5 $\mu \times$ 3·5–4 μ. Brug (1922) observed cysts of *Waskia* [=*Embadomonas*] *belostomae* in the contents of the rectal diverticulae of *Belostomum indicum*. They measured 6 $\mu \times$ 3–4 μ.

In the strain of *Embadomonas* from the frog, cultivated on egg slopes covered with serum-Ringer, cysts appeared in large numbers in the early subcultures, but after the 12th subculture no more appeared though I kept the strain for months. I also obtained large crops of cysts in a strain from the toad. The cysts were colourless, oval, or slightly pointed at one end. The average measurement was $6\mu \times 4{\cdot}5\mu$. Little could be seen of the internal structure in the living cyst or in cysts stained with iodine, but when stained with iron haematoxylin the internal structures could be seen. Several individuals were found (fig. 23) rounded—but without a cyst wall. In these the nuclear peripheral chromatin was normal, but a central karyosome was present. The pair of basal granules was quite obvious, as was part of the fibril surrounding the cytostome. The cytostomial flagellum could be detected but the anterior flagellum was not always plain. Cysts still rounded, but with a cyst wall, were found. In these (figs. 24 and 26) the pair of basal granules were very definite, the shape of the nucleus was almost normal, and the course of the two flagella could be followed in some though not in all. At this stage a colourless area frequently appears in the cyst. Later (fig. 25) the karyosome increases in size and the peripheral chromatin elongates. The cyst changes a little in shape as it matures, and becomes pointed at one pole; the elongated peripheral chromatin (figs. 27 and 28) stains more intensely, and the karyosome increases greatly in size. A light area is always visible, but I am uncertain as to its significance. A darkly staining fibril, which I interpret as the vestige of the lip of the cytostome, is invariably present. Other fainter fibrils are sometimes discernible and these, I conclude, are the flagella—as in *E. blattae*. The pair of prominent basal granules are a diagnostic feature of the cyst of this *Embadomonas*.

The cyst of the *Embadomonas* from the salamander (fig. 30) is similar to that of *Embadomonas* from frogs and toads. The structures of these Embadomonads are, in all particulars, so similar that I have no reason to believe that there is any specific difference. I have never observed any cyst whose structure suggested that division occurs during encystment, nor that "double" individuals encyst.

The cysts of this species differ very much from those of *E. alexeieffi* and *E. agilis* described by Mackinnon, since in these latter species there is no elongation of the nucleus. Wenyon and O'Connor (1917) describe a central karyosome and an elongation of the nuclear membrane in the majority of the cysts of *Waskia* [= *Embadomonas*] *intestinalis*. Dobell and O'Connor (1921) describe the cysts of this species from man as uninucleate, but believe the looped thread to be the remains of the fibril supporting the cytostome, with this Jepps (1923) agrees. Collier and Boeck (1926) describe, in the cyst of *E. cuniculi*, a nucleus, central in position, with a definite membrane and karyosome which may be connected by a rhizoplast to a centrosome on the nuclear membrane. The blepharoplasts lie at the anterior end of the cyst and from one of them runs the cytostomial fibre and a rhizoplast to the centrosome. The

dual nature of the blepharoplast is not visible in their drawing. Broughton-Alcock and Thomson (1922) in the cysts of *Embadomonas intestinalis* interpret the elongated, darkly staining body as the cytostome, and the granule, or granules, inside (*i.e.* the karyosome according to my interpretation) as the nucleus. They do not describe or figure in the cysts the pair of blepharoplasts.

I propose to call the *Embadomonas*, which I have studied from *Rana temporaria*, *Bufo vulgaris* and *Salamandra maculosa*, *E. dobelli* because Mr Dobell first saw and figured the cysts (Dobell 1909) though he believed them to belong to another flagellate.

Table I.

Table of measurements of species of Embadomonas *described up to the present.*

Species	Size of flagellate in microns	Host	Author
E. agilis	4–11 × 1·5–3	Larvae of *Trichoptera* and *Tipula*	Mackinnon (1915)
E. alexeieffi	7–16 × 5–9	Larvae of *Tipula*	,,
Waskia intestinalis [= *E. intestinalis*]	4–9 × 3–4	Man	Wenyon and O'Connor (1917)
E. intestinalis	5–6 × 3–4	,,	Dobell and O'Connor (1921)
,,	3·5–9 × 1·5–6	,,	Broughton-Alcock and Thomson (1922)
E. sinensis	10–14 × 4·2–7	Man (Chinese)	Faust (1923)
Waskia wenyoni [= *E. wenyoni*]	14 × 12	*Cebus caraya*	da Fonseca (1920)
E. cuniculi	7·5–13 × 5·5–9·5	Rabbit	Collier and Boeck (1926)
E. bradypi	4–6·5 × 1·6–3·2	*Bradypus griseus griseus*	Hegner and Schumaker (1928)
E. caviae	4–7 × 2–4	Guinea-pig	,,
E. ovis	4·5–6·1 × 2·7–4·7	Sheep	,,
E. blattae n.sp.	6–9 × 5–7	*Blatta orientalis*	Bishop (1931)
E. dobelli n.sp.	6–11 × 4–8	*Rana temporaria*, *Bufo vulgaris*, *Salamandra maculosa*	,,

V. The problem of the "cyst" of *Trichomonas batrachorum*.

The question whether an encysted stage occurs in the life-history of *Trichomonas* has been raised many times in the literature. In the early accounts much confusion arose from the wrongful identification of *Blastocystis* (an organism occurring in the intestine of most animals) as the cyst of *Trichomonas*. Alexeieff (1911) cleared up the matter, and now *Blastocystis* can be cultivated—only too readily!—on media used for cultivating intestinal protozoa, and obtained in "pure" culture if necessary. Many descriptions of cysts of *T. hominis* have proved erroneous. In spite of a wealth of material from cases infected with *T. hominis* Wenyon and O'Connor (1917), Dobell and O'Connor (1921), Pringault (1920) and others have failed to find cysts. Hogue (1921 and 1922) reports that none appeared in her cultures of *T. hominis*. I have kept cultures of *T. hominis*, *Trichomonas* from *Macacus rhesus* and *M. nemestrinus* and have made and examined a great number of preparations without finding an encysted stage. Rounded, degenerating stages (Bishop 1931, fig. 40)

appeared in old cultures, which might lead one to suspect a cyst, but careful examination showed all stages in degeneration.

Encysted stages of *T. caviae* have been described (Galli-Valerio 1900, 1903; Brug 1917; Kuczynski 1914; Wenyon 1926), whilst rounded resistant forms have been described in *T. muris* by Wenyon (1907), Mayer (1920), and Wenrich (1921).

With regard to *T. batrachorum*, Grassé (1926) described a process of encystation in which there was a gradual absorption of the axostyle, a shedding of the flagella, and later of the undulating membrane. He describes an oval cyst measuring 8–10μ × 5·5–7μ. The nucleus of the fully developed cyst, according to his account, is ellipsoidal, without a karyosome, and with a rounded parabasal body and a single blepharoplast lying close beside it. A comparison of his figures with those of *T. caviae* by the previously cited authors reveals a great difference in general appearance.

Dobell (1909), in his study of the intestinal protozoa of frogs and toads, described some cysts from faeces which he believed to be the cysts of *Eutrichomastix batrachorum* and *Trichomonas batrachorum*. He gives their average dimensions as 6·5μ × 5μ. He figures (figs. 14, 15, 26, 27 and 28) an elongated nucleus and karyosome, and a pair of minute granules. Wenyon (1926) remarked upon the similarity between Dobell's figures of the cysts of *E. batrachorum* and *T. batrachorum* and the cysts he found in a culture of *Embadomonas* from the frog.

Mr Dobell has discussed the matter with me. He has examined two of my preparations of the cysts of *Embadomonas* and compared them with his own preparations of smears of faeces from frogs. He placed his preparations at my disposal and I have examined them carefully. We are both now agreed that the cysts which, years ago, he believed to be the cysts of *E. batrachorum* and *T. batrachorum* are really the cysts of *Embadomonas*. In searching his preparations we found some faintly stained flagellate-forms of *Embadomonas* present among the many Trichomonads.

The difficulties in attempting to determine the cyst of a particular species, if working upon intestinal contents of a host which is infected with a number of species of intestinal protozoa, and which may contain cysts of free-living forms swallowed in food, are enormous. *T. batrachorum* is a very common parasite of frogs and toads and frequently is present in large numbers. If infection is brought about by cysts it seems justifiable to expect the cysts to be numerous. I have examined gut contents swarming with *T. batrachorum*, but have failed to find cysts which I could assign to that species. Experience in making examinations of faeces from monkeys had taught me that cysts occurred sporadically and that several daily examinations might be negative for them. I therefore chose a healthy toad which was infected with *T. batrachorum*, and kept it isolated and well fed. I repeatedly examined faeces from it, but though I found many Trichomonads no cysts appeared.

I concluded that the safe diagnosis of cysts would result from obtaining

them from pure cultures of *T. batrachorum*. Past experience had also demonstrated that forms which are found to encyst readily in culture (*e.g. Entamoeba histolytica, E. coli, Chilomastix mesnili, C. caulleryi*) will not always do so. Of two strains isolated from the same host on the same day and grown in the same kind of medium one may produce cysts whereas the other may not. Even two subcultures from the same primary culture may behave differently in this respect. Strains which encyst well for some generations after isolation sometimes later lose their power to do so (*e.g. Embadomonas*, p. 295). If therefore I isolated *one* strain of *T. batrachorum* and cultivated it for a number of months without obtaining cysts it would prove nothing; but the fact that I have cultivated (Bishop 1931) one strain for five months, three strains for over two months and a large number of strains for shorter periods without finding a single cyst does seem to me to provide some evidence against the existence of cysts in this species.

Summary.

1. *Embadomonas blattae* a new species from the hind gut of *Blatta orientalis* is described. The flagellate measures 6–9μ × 5–7μ and the cysts 5–6μ × 4–5μ.

2. An *Embadomonas* from *Rana temporaria, Bufo vulgaris*, and *Salamandra maculosa* has been cultivated and described. I propose to call it *E. dobelli*. It differs from other known species of this flagellate in shape, and in absence of a definite karyosome. The flagella and their basal granules are duplicated first at division, and a centrodesmose is formed between one of each pair of basal granules. The cytostome disappears and new ones are reorganised after nuclear division is completed. Definite chromosomes were never seen. Double individuals and occasionally individuals with three nuclei were seen.

3. Cysts of *E. dobelli* were obtained in culture. They are uninucleate. A karyosome is formed; the peripheral chromatin of the nucleus stains intensely and becomes elongated. The lip of the cytostome can often be distinguished, and sometimes the flagella. These cysts are the same as those described originally by Dobell as cysts of *Eutrichomastix batrachorum* and *Trichomonas batrachorum*.

4. Cysts of *Trichomonas batrachorum* have never been found in faeces or in cultures.

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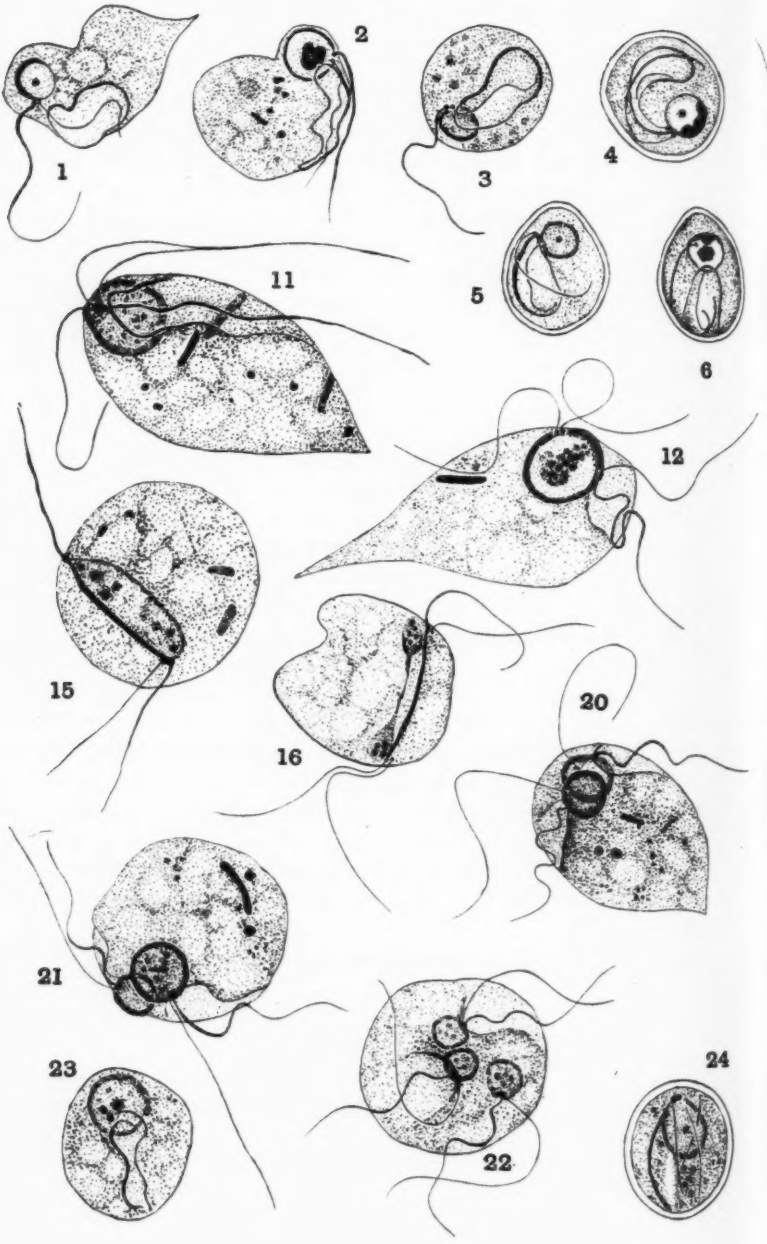
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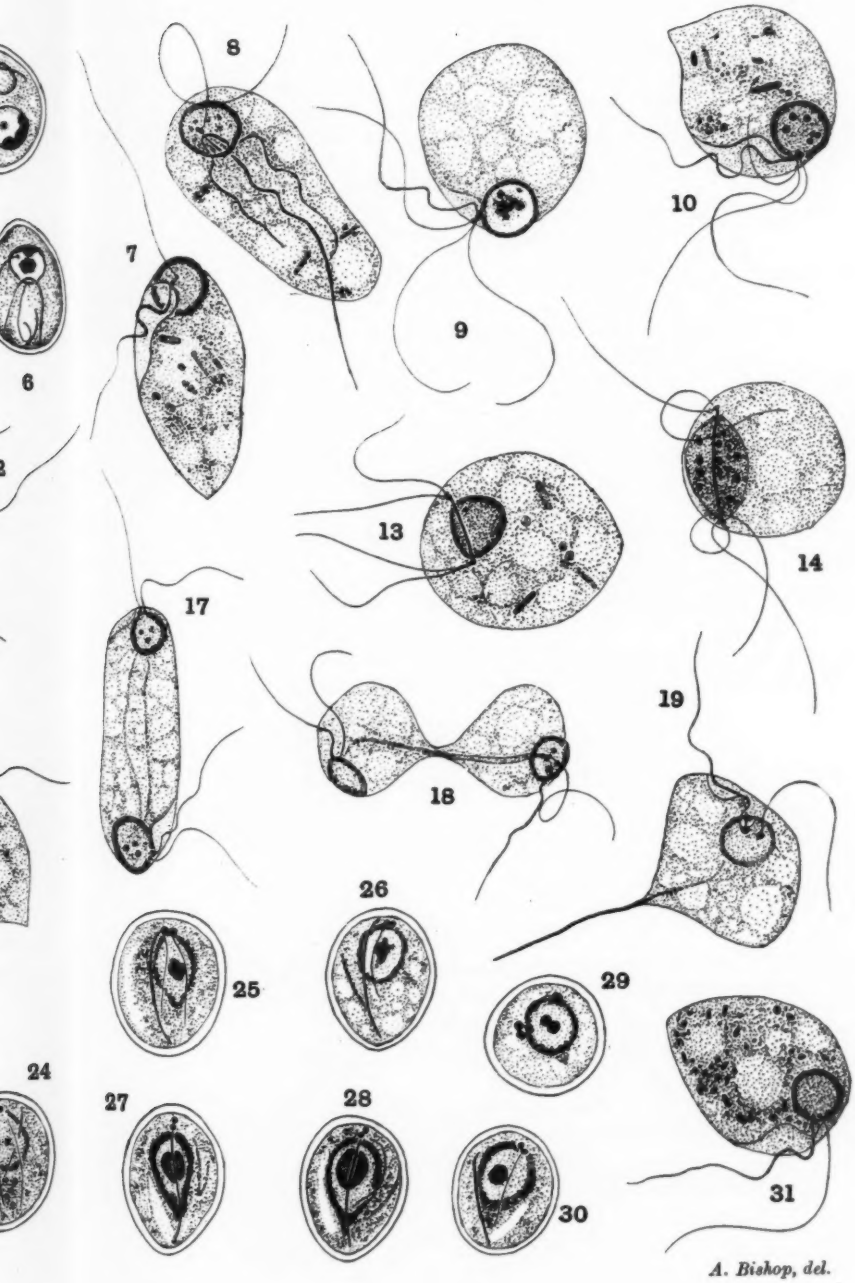
PLATE XII



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EXPLANATION OF PLATE XII.

The figures have all been drawn with an Abbé camera lucida on a Leitz binocular stand, with a $\frac{1}{12}$ in. A fluorite oil immersion lens at an approximate table magnification of 3300 diameters.

Figs. 1–6. *E. blattae.*

1, 2. *E. blattae* showing nucleus, basal granules, flagella and cytostome.

3. Precystic individual.

4–6. Cysts.

Figs. 7–29. *E. dobelli.*

7–8. Individual showing nucleus, cytostome, basal granules and flagella.

9–11. Individual preparing to divide, four flagella.

12. The pairs of flagella have moved to opposite sides of the nucleus.

13. Same, centrodesmose shown.

14. Metaphase.

15. Anaphase.

16. Telophase; shows basal granules at either end of centrodesmose and at base of flagella.

17. Later telophase. Cytostomial flagella are becoming differentiated.
18. Individuals about to divide.
19. Daughter separated.
20. Double individual.
21. Same, with one nucleus larger than the other.
22. Trinucleate individual.
23. Precystic individual.
24. Early cyst.
25. Same.

26–28. Cysts.

29. Cyst viewed from pole.
30. Cyst of *Embadomonas* from *S. maculosa*.
31. Flagellate stage of *Embadomonas* from *S. maculosa*.

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DO PENETRATING NEMATODE LARVAE ASSIST BACTERIAL INVASION FROM THE BOWEL?

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Historical.

That parasitic worms may induce bacterial invasion from the intestine has frequently been asserted in explanation of the sporadic occurrence of some bacterial and virus diseases of animals. Weinberg, in 1907, gave an extensive review of the literature dealing with inflammatory conditions of the intestine, due to bacterial invasion associated with parasitic worms. He also gave an account of some original clinical observations. He concluded that, by biting and boring into the intestinal wall, or by wounding it with hooks and suckers, the majority of helminths favour the penetration of microbes, these being carried on the exterior and in the intestine of the worms. In one case, where a fatal septicaemia was associated with the presence of a single individual *Trichocephalus*, the author's conclusion, that even isolated worms are very dangerous, appears to be rather strained. Records of clinical observations of this kind frequently appear in the literature, but, as a general rule, the evidence showing the agency of the worm is very slender and amounts to little more than the observation of the presence of worms in animals suffering from some bacterial disease.

Hutyra and Marek refer to a paper by Lignières (1898)—inaccessible to us—in which haemorrhagic septicaemia of sheep is associated with verminous bronchitis and gastritis, the infection supposedly being assisted by the injuries to the mucous membrane inflicted by the worms. These authors, however, state that Lignières' view must be considered an extreme one, as haemorrhagic septicaemia frequently occurs in the absence of parasitic worms.

Aynaud (1926) reports the presence of nematode larvae in blackleg and bradsot tumours. In view of the epizootiology of the diseases, he considers it possible that blackleg and bradsot may be inoculated by worms.

Basset and Carré (1907) demonstrated that the dog's intestinal wall (although an effective barrier against the bacterial inhabitants of the bowel contents) could be rendered permeable to the *Coccus* of Mathis through previous injury with drastic purgatives. From this Carré (1928) argues that similar effects may be produced through lesions caused by *Ascaris* in the dog, and other intestinal parasites in sheep. In a series of experiments he tried to reproduce Aynaud's "Botryomycosis of sheep," by feeding to them large

quantities of the causal organism. The first attempt, on eleven lambs which had been born and reared at the laboratory, was not successful. In a second attempt, however, one of six lambs became infected. These six animals were known to be harbouring intestinal coccidia and worms, and it was thought that these parasites may have been responsible for the successful infection.

In 1898, Besnoit and Cuillé published a short account of a serious epizootic occurring in the south-west of France among sheep and cattle. Deaths were chiefly due to fascioliasis, but a haemorrhagic septicaemia was in many cases associated with the helminthic infection. The authors concluded that internal parasitism, by its weakening action and also sometimes by causing local injury, brings about a condition eminently predisposing animals to microbial infections in general.

Basset and Moulin (1930) give a short account of an epidemic of a fatal septicaemia among mules due to *Bacillus coli* which they consider to have been inoculated by strongyloid worms in the bowel.

Von Bülow (1929) describes a case of inactive pulmonary tuberculosis which, as strongly suggested by the circumstances, was reactivated by the passage of hookworm larvae through the lungs. He thinks that these larvae may sometimes be the means of carrying tubercle bacilli from infected faeces to a new host.

In a series of experiments on infectious enteritis in swine, Biester and Murray (1928) found that the lesions produced in subnormal subjects were much more advanced than those produced in apparently healthy swine. They state that the stunted growth of these pigs was apparently due to *Ascaris lumbricoides* and presumably this parasite may have played a direct part in the production of the advanced lesions.

The association of the liver fluke with black disease in sheep seems to be well established. The worms in this condition are, however, concerned not with the introduction of *B. oedematiens*, but in the production of suitable conditions for the growth of bacilli already present in the liver.

According to the English summary of a paper by Kawanishi (1929) the entry of typhoid bacilli into the blood stream of a guinea-pig was experimentally induced by placing infective hookworm larvae in an emulsion of the bacilli on the skin, the bacilli being recoverable from various organs within an hour. It was found that many bacteria adhered firmly to the surface of the larvae, but that none could be found within the larvae, owing, it is stated, to the presence of a "bactericidal ferment."

Malvoz and Lambient (1918) state that they produced a generalised tuberculosis infection in a guinea-pig by placing on a shaved area of the skin a suspension of ancylostome larvae together with sputum containing many bacilli. They also state that when a *B. anthracis* culture was used in the same way infection was very readily produced. These authors express the opinion that in addition to the direct inoculation of a virus such as was experimentally obtained by them, the ancylostome larvae may act in a more important way

as carriers of viruses of disease from the faeces of infected persons to new hosts.

De Blieck and Baudet (1920) carried out some controlled experiments in which it was shown that the larvae of *Strongyloides westeri*, when placed in an emulsion of bacteria on the skin of laboratory animals, were capable of introducing the micro-organisms into the animal body. Positive results were obtained in this way with the organism of fowl cholera in rabbits, anthrax in guinea-pigs, and swine erysipelas in mice.

For a long time *Heterakis* has been associated with enterohepatitis of turkeys, and Tyzzer (1926) has shown that this worm actually plays the part of intermediate host in the life cycle of the causal organism.

Paralysis and leucaemia of fowls, both supposedly caused by a filter passing virus, have by several authors been associated with the tapeworm *Davainea proglottina*. In the report of a series of observations on the pathology of fowl leucaemia Bayon (1929) says that the analysis of a particular outbreak of the disease which he studied suggests that massive infestation with *D. proglottina* brings about a receptive condition of the bone marrow, the cestode in this way acting as a preparatory cause.

The preceding short survey of the relevant literature shows that many observers have regarded parasitic worms in general, and nematodes in particular, as agents in the propagation of bacterial diseases. Only a very few controlled experiments have, however, been undertaken to verify or disprove this hypothesis.

Authors' experiments.

In the experiments here reported, we have tried to ascertain whether nematode larvae which penetrate the intestinal wall are able to assist bacteria already present in the lumen of the intestine to invade the body of the host animal.

Nematode larvae.

Larval ascarids are among the best known of boring nematode larvae, and as ascarids are of very common occurrence in some of the domesticated animals, and the larvae are known readily to penetrate the intestinal mucosa of laboratory animals, they seemed to be admirably suited to these experiments.

Ascaris larvae have the added advantage that evidence of their having penetrated the bowel wall is readily obtained by examining the lungs of the test animal.

Ascaris lumbricoides of the pig was used because it is easy to obtain in large numbers. For the first experiment the eggs were incubated for eight weeks at 26° C. in a 2 per cent. formalin solution and so the number of eggs given in the dose could be counted. For the other experiments very large numbers of eggs were similarly incubated in coarse-grained animal charcoal, moistened with a 2 per cent. formalin solution. This method was found very

convenient as thorough aeration could be easily obtained, and although the number of eggs in a dose could not be counted, relative amounts could be weighed out as required, and the culture be more easily handled.

The dose of infective *Ascaris* eggs given to the experimental animals was a large one, in order to make the test as severe as possible. Several of the experimental animals died of *Ascaris* pneumonia as a result, but death from this cause did not occur until all chances of bacterial infection had passed. Except when hatched larvae were given, in every case the lungs of the experimental animal were found to be very badly affected, thus giving evidence of the large numbers of larvae which had penetrated the intestinal mucous membrane.

With reference to the successful results reported by Malvoz and Lambient (1918), the photo-micrographs illustrating the paper show that a very great concentration of larvae was used. It seems not unlikely, in these circumstances, that a reaction in the experimental patch of skin might be induced to which the resulting infection could be traced, rather than to the mere carrying in of the bacteria by the larvae. If such a concentrated action of *Ascaris* larvae in the alimentary canal of experimental guinea-pigs could be induced, a similarly successful infection might result, but this would be an experimental test far removed from what is likely to occur in nature. The same applies to the results of De Blieck and Baudet (1920).

Bacillus anthracis.

B. anthracis at first appeared to be a suitable organism for this purpose when given to guinea-pigs.

Although it is generally agreed that guinea-pigs are resistant to anthrax infection *per os*, as shown by Besredka (1921), Balteano (1922), Grierson (1929) and others, their resistance is by no means absolute. Boquet (1924) produced infection by the injection of cultures into the tongue, and concluded that the mucous membrane can act as a receptor organ just as the skin does. Grierson (1929), in two instances in a series of guinea-pigs produced infection by feeding cultures of *B. anthracis* in bran; he says: "It is of interest to note that Pasteur was able to infect animals by the oral administration of anthrax bacilli only when some sharp material such as bristles, which injured the mucosa, were mixed with the food." An observation of Basset and Denizot (1929) also supports this view. They reported a sudden outbreak of anthrax in sheep following the ingestion of nitrate of soda, and ascribed it to the lowered resistance of the intestinal wall resulting from the violent irritation caused by the salt.

If the intestinal wall is not an absolute defence against infection with *B. anthracis* it appeared to us that possibly the injuries caused by penetrating nematode larvae might be sufficient to overcome the protective mechanism. Moreover, anthrax is one of those diseases whose sporadic occurrence might be explained by the agency of parasitic worms.

First experiment.

In this experiment, twenty-four guinea-pigs of 250–350 grm. weight were fed, through a pipette, varying doses of a suspension of vegetative forms of *B. anthracis*. Twelve of these guinea-pigs also received doses of infective *Ascaris lumbricoides* eggs, the other twelve being kept as controls. As in nine days' time anthrax infection had not appeared, it was decided to repeat the doses.

The emulsion of *B. anthracis* was prepared from a two days' old growth of nutrient agar; its virulence was proved by inoculating a scarified area of skin of a test guinea-pig which died of anthrax after two-and-a-half days.

Microscopical and cultural examinations were made of all guinea-pigs which died, but in no case could the presence of anthrax bacilli be demonstrated. The seven guinea-pigs which succumbed died of *Ascaris* pneumonia, and the five which were killed showed extensive lung lesions due to the larvae.

Table I.

	Date of first dose 4. vi. 29			Date of second dose 13. vi. 29		
No. of guinea-pig	Quantity of *B. anthracis* emulsion in c.c.	Number of *Ascaris* eggs	Results after nine days	Quantity of *B. anthracis* emulsion in c.c.	Number of *Ascaris* eggs	Results 1929
29	0·1	7000	Nil	0·1	42,000	Killed 18. vi.
30	"	"	"	"	"	Died 16. vi.
31	"	"	"	"	"	Killed 18. vi.
32	0·5	14,000	"	0·5	"	Killed 18. vi.
33	"	"	"	"	"	Died 13. vi.
34	"	"	"	"	"	Died 14. vi.
35	0·1	"	"	0·1	"	Died 15. vi.
36	"	"	"	"	"	Killed 18. vi.
37	"	"	"	"	"	Killed 26. vi.
38	0·5	7000	"	0·5	"	Died 25. vi.
39	"	"	"	"	"	Died 25. vi.
40	"	"	"	"	"	Died 21. vi.
41	0·1	Nil	"	0·1	Nil	Lived
42	"	"	"	"	"	"
43	"	"	"	"	"	"
44	"	"	"	"	"	"
45	"	"	"	"	"	"
46	"	"	"	"	"	"
47	0·5	"	"	0·5	"	"
48	"	"	"	"	"	"
49	"	"	"	"	"	"
50	"	"	"	"	"	"
51	"	"	"	"	"	"
52	"	"	"	"	"	"

Second experiment.

Having failed to produce anthrax by the administration of the vegetative forms along with *Ascaris* eggs containing infective larvae, we next tried anthrax spores and *Ascaris* eggs, given in daily doses over a period of ten days.

The anthrax spores were taken from agar plates which had been incubated for five days at 37° C., and kept at room temperature for a further two days. Stained smears showed a very high percentage of sporulating forms. The infective *Ascaris* eggs were incubated in coarse animal charcoal.

In this and the previous experiment described, sixty guinea-pigs received large doses of virulent anthrax bacilli, both vegetative forms and spores, but in no instance were we successful in producing anthrax. Besredka (1921), Balteano (1922) and Grierson (1929) have shown that it is possible for virulent anthrax bacilli to circulate in the blood without producing disease, and that anthrax will not develop from this condition unless the skin, which Besredka regards as the receptor organ, is injured in some way. In the light of these observations, so that every opportunity might be given for the development of the disease should the bacteria have been introduced into the circulation from the intestine, we snicked the ears of the thirty-six guinea-pigs on the ninth, tenth and eleventh days in the second experiment.

Table II.

Period of feeding 25. ix. 29 to 5. x. 30	Anthrax spores: 2 million daily	Large dose of *Ascaris* larvae daily	Results 1929
12 guinea-pigs	Nil	+	One killed 14. x. Showed extensive lesions of *Ascaris* pneumonia
12 guinea-pigs	+	+	One killed 14. x. Showed extensive lesions of *Ascaris* pneumonia
24 guinea-pigs	+	Nil	One died 30. ix. Autopsy findings nil One died 5. x. Autopsy findings nil One died 12. x. Autopsy findings nil Stomach inflamed Two killed 14. x. Autopsy findings nil

From the negative results of the two experiments we conclude that large numbers of *Ascaris* larvae penetrating the intestinal mucosa in the presence of anthrax bacilli and spores are not able to set up infection in the guinea-pig. But, in view of the completely negative results obtained, and as the conditions for successful infection with this bacillus are peculiar, we searched for another and more promising organism.

Bacillus suipestifer.

For the next experiment we wished to try some organism which definitely would produce infection by feeding. We could then fix its minimum infective dose when given alone, and proceed to ascertain whether that dose could be lowered by giving large numbers of penetrating nematode larvae at the same time.

B. suipestifer appeared to answer requirements, being highly pathogenic for rabbits when given subcutaneously in small doses, while infection can be readily induced by ingestion. A strain of *B. suipestifer*, kindly supplied by T. M. Doyle of this laboratory, was found to be highly pathogenic for rabbits by the subcutaneous route, and by the mouth if the dose was sufficiently

large. Preliminary tests showed that $\frac{1}{25}$ c.c. of a twenty-four hours' broth culture of this strain, given by the mouth, would kill rabbits consistently, and that $\frac{1}{75}$ c.c. would kill only occasionally.

Third experiment.

In this experiment with *B. suipestifer* we tried to produce infection by giving large doses of *Ascaris* eggs to rabbits for three days, accompanied on the second day by a dose of $\frac{1}{75}$ c.c. of emulsified *B. suipestifer*, so as to be sure that active larvae would be present in the gut when the bacilli were introduced. The dose of infective *Ascaris* eggs was the same as in Experiment II (*q.v.*). Details of the experiment are given in Table III.

Table III.

Composition of experimental feed	No. of rabbit	Results
Ascaris eggs and *B. suipestifer*	A 1	Two died 11 days after first feed; autopsy showed verminous pneumonia. Bacteriological examination negative.
	A 2	
	A 3	
	A 4	The three other rabbits remained healthy
	A 5	
B. suipestifer	D 1	Remained healthy
	D 2	
	D 3	
	D 4	
	D 5	
Ascaris eggs	G 1	One was killed 9 days after the first feed. Autopsy showed extensive lesions of *Ascaris* pneumonia. Many larvae were recovered
	G 2	
B. suipestifer subcutaneously	H 1	Died four days after inoculation, and *B. suipestifer* was recovered

Fourth experiment.

In this experiment the dose of *B. suipestifer* was increased to $\frac{1}{50}$ c.c. of a twenty hours' broth culture and that of *Ascaris* eggs containing infective larvae to three grams of culture; while a mixture of bacterial emulsion with hatched *Ascaris* larvae (obtained from infected lungs) was also tried.

Details of the experiment are given in Table IV.

The dose of *Ascaris* eggs containing infective larvae was 3 grm. of a charcoal culture. The hatched larvae had been collected by means of Baermann's apparatus from infected rabbits' lungs, and were kept in contact with the bacterial emulsion for thirty minutes before being given to the rabbits.

DISCUSSION.

The arrangement of these experiments ensured that *B. anthracis* and *B. suipestifer* were present in the alimentary tract of susceptible laboratory animals simultaneously with large numbers of *Ascaris* larvae. The number of larvae which penetrated the mucosa was evidently great, as many of the animals died of *Ascaris* pneumonia. There seems to be every reason to suppose that the larvae which penetrated the mucous membrane would carry with them some of the pathogenic bacteria, but we were not able to find any indication

Table IV.

Rabbit No.	Route of inoculation	*B. suipestifer* (c.c.)	*Ascaris* eggs	Results		Autopsy findings
1	*Per os.*	+	+	Died day	11	All deaths were due to *Ascaris* pneumonia.
2	,,	+	+	,,	9	
3	,,	+	+	Lived		
4	,,	+	+	Died day	10	Attempts to recover *B. suipestifer* failed
5	,,	+	+	,,	10	
6	,,	+	+	,,	10	
7	*Per os*	–	+	Died day	10	Deaths due to *Ascaris* pneumonia
8	,,	–	+	,,	11	
9	,,	–	+	,,	13	
10	Subcutaneous	0·002	–	Died day	6	*B. suipestifer* recovered from both cases
11	,,	0·02	–	,,	6	
12	*Per os*	+	–	Lived		*B. suipestifer* recovered from all four cases
13	,,	+	–	,,		
14	,,	+	–	Died day	13	
15	,,	+	–	,,	7	
16	,,	+	–	,,	8	
17	,,	+	–	,,	17	
18	*Per os*	–	Hatched larvae	Killed day	15	No lesions caused by *Ascaris*
19	*Per os*	+	+	Lived		*B. suipestifer* recovered
20	,,	+	+	,,		
21	,,	+	+	,,		
22	,,	+	+	Died day	8	
23	,,	+	+	Lived		
24	,,	+	+	,,		
25	Subcutaneous	0·002	+	Died day	7	*B. suipestifer* recovered from both cases
26	,,	0·02	+	,,	7	

that they helped to bring about infection. As it is difficult to produce anthrax in guinea-pigs by feeding with cultures, this organism may not have been a suitable one for the experiments, and no general conclusions can be drawn from that part of the work. *B. suipestifer* seems, on the other hand, to have been suitable, and we feel justified in coming to some conclusions. The most likely explanation for the apparent failure of the *Ascaris* larvae to assist infection with *B. suipestifer* appears to be that the number of bacteria entering the tissues with the larvae is insignificant, compared with the number which enter without their aid: in which case our experiments would not be able to indicate the action of the larvae.

Conclusions.

1. It is difficult to set up anthrax in the guinea-pig by oral administration of infective material. This confirms the findings of other workers.

2. The penetration of the guinea-pigs' intestinal mucosa by *Ascaris* larvae in the presence of anthrax bacilli or spores, does not induce anthrax infection.

3. *Ascaris* larvae penetrating the rabbit's intestinal mucosa, in the presence of *B. suipestifer*, play no part in the invasion of the body by the bacteria.

4. Although it is possible that larger wounds, caused by some mature

worms in the alimentary tract, may represent a breach in the defence against pathogenic bacteria, the wounds caused by burrowing nematode larvae are apparently insignificant, and the number of pathogenic bacteria carried by the larvae into the tissues is too small for these minute worms to play any part in the invasion of bacteria from the bowel.

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ON THE STRUCTURE AND LIFE HISTORIES OF BRITISH FRESH-WATER FURCOCERCARIAE.

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(With 3 Figures in the Text.)

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I. Introduction.

Very few species of British fresh-water furcocercariae have been recorded. One unnamed species and probably new is described by Hesse (1923) from *Limnaea peregra* Müll. from Loch Lubnaig and Loch Vennachar, Scotland. Brown (1926) has recorded three species from central England; one as *Cercaria fissicauda* La Val. from *L. stagnalis* L., and *C. macrosoma* n.sp. and *C. micromorpha* n.sp. from *L. stagnalis* L. and *L. peregra* Müll. respectively. Nothing is known of the further development of any of these species.

In this paper three species are described, two of which appear to be new. I have also been able to trace the development of two of the species into the corresponding *Tetracotyle* (larval Holostome) stages, one of which is also new. The material investigated was obtained from Morton Loch, North Fifeshire. The work was carried out in the Department of Natural History, University College, Dundee, under the late Prof. Gemmill, F.R.S., during my tenure of a Research Maintenance Allowance from the Department of Scientific and Industrial Research (1923–5) and a Carnegie Research Scholarship (1925–6).

In a previous paper (Harper, 1929) it was pointed out that the identification of cercariae is difficult owing to the lack of detailed descriptions of the majority of named European forms. In the present case I have been able, by means of direct animal experiment, to allocate one species to a known adult, and in the

absence of further evidence as regards their life histories I have referred to the others as *Cercaria* F.1 and F.2 respectively. In all cases I have tried to indicate the European species falling nearest to my description. Lühe's key (1909, pp. 203–4) based on the characteristics of the tail does not help much in this connection, while the recognition of cercariae according to the hosts in which they develop is of little use, since it is evident that more than one species of mollusc can harbour the same cercaria. For recognising the species described here and for comparison with others I have relied mainly on the number and position in the body of the cercaria, of the so-called salivary gland cells which appear to be constant within the species. This method has also been used by Szidat (1924*a*) and by others for the recognition of human Schistosome cercariae.

II. Previous work on the development of larval holostomes.

Although many continental furcocercariae have been known for a considerable time it is only within recent years that research has been directed towards the elucidation of their life histories. This work has shown that certain of these forms develop into definitely recognisable Holostome larvae of the type *Tetracotyle* and *Diplostomum*. *Tetracotyle* species occur abundantly in certain molluscs, leeches and vertebrates and are provided with a definite cyst wall. In *Diplostomum*, examples of which are found in the eyes, brain and spinal cord of fishes and amphibians there is no cyst wall, the larvae being free moving.

Linstow (1877) and Brandes (1888, 1891) were of the opinion that the encysted *Tetracotyle* arose more or less directly from the egg, with the elimination of the sporocyst and cercaria stages. Lutz (1921) seems to have been the first to point out the connection between furcocercariae and Holostome larvae. Ruszkowski (1922) traced the development of a fork-tailed cercaria from *Planorbis vortex* L. into *Hemistomum alatum* Dies. Szidat (1924*a*, *b*) showed that *Cercaria* A. from *Limnaea palustris* Müll. develops into *Tetracotyle typica* Dies. the larva of *Strigea tarda* Steenstrup, and that *Cercaria* C. from *L. ovata* Drap. and *L. stagnalis* L. develops into *Diplostomum volvens* v. Nordm. the larva of *Hemistomum spathaceum* Rud. (Braun, 1894*a*).

From the work of Szidat it is also evident that certain species of furcocercariae are capable of continuing their development into the encysted *Tetracotyle* in fresh-water leeches. *Tetracotyle typica* de Fil., previously recorded only from certain fresh-water molluscs, has been obtained by Szidat (1929, p. 139) from the leeches *Herpobdella atomaria* Carena and *Haemopis sanguisuga* L., and by feeding experiments using ducklings as final hosts he has shown it to be the larva of *Cotylurus cornutus* Rud. A new *Tetracotyle* from the leech *Herpobdella atomaria* Carena is also described by the same author (*loc. cit.* p. 143) and proved by him to be the larva of *Apatemon gracilis* Rud. He was not successful, however, in ascertaining the cercaria belonging to this life cycle.

In this connection it is interesting to note that isolated examples of mobile phases of a *Tetracotyle* were found by v. Linstow (1893), on the skin of leeches, but never in the body. These phases measured 0·35 × 0·26 mm., but their structure was not definitely made out. Braun (1894*b*) found dozens of encysted *Tetracotyle* in *Nephelis* sp., but their identity was not established.

III. Description of parasites.

Observations on the life history of Strigea tarda *Steenstrup.* (Fig. 1 A–E.)

It has now been definitely proved that *Strigea tarda* Steenstrup develops from a furcocercous larva produced in sporocysts in a fresh-water mollusc and that it has an encysted stage (*Tetracotyle typica* Dies.) in the same host. The development of *T. typica* Dies. into the adult Holostome is first recorded by Ercolani (1881) who, however, referred the larva in error to *Holostomum erraticum* Rud. Lühe (1909) gives the adult as *Strigea tarda* Steenstrup which occurs in the intestine of various ducks. Mathias (1924) and Szidat (1924*a*) have repeated the feeding experiments of Ercolani with *T. typica* Dies. and both these authors obtained the adult *S. tarda* Steenstrup. Szidat (*loc. cit.*) has in addition proved that *T. typica* Dies. itself develops from *Cercaria* A. a fork-tailed larva from *Limnaea palustris* Müll.

The following observations on the life history of *S. tarda* Steenstrup are presented as a connected detailed account of the various stages in the life cycle, as they occur in Scottish hosts. This seems desirable not only since the mollusc intermediary in this country is different from that on the continent, but because it is evident that in certain fresh-water molluscs there exist encysted *Tetracotyle* species which in the absence of detailed examination may be mistaken for *T. typica* Dies. Further, it is reasonable to suppose that such encysted forms develop from closely allied species of furcocercariae. Accordingly it is hoped that the following account will provide a recognisable description of the various stages in the life cycle.

The Sporocysts occur in the liver and gonads of *Limnaea peregra* Müll. They are white thread-like structures, usually very numerous and exceedingly involved. In all heavy infections liver and gonads are quite destroyed. An individual sporocyst may reach a length of 4 mm. with an average breadth of 0·15 mm. The whole sporocyst is active, especially the anterior end which projects from the liver tissue. A birth pore is present about 0·1 mm. from the anterior end.

The Cercaria is translucent and flattened dorsiventrally. The tail is stout and forked distally. The lengths of the component parts of the larva are as follows: body 0·14–0·16 mm., median caudal stem 0·18 mm., furci 0·18–0·2 mm. Anteriorly the body is very contractile and bears numerous transverse rows of minute spines, the extreme anterior rows entering the buccal cavity. There are two suckers, an ovate oral sucker the walls of which

are protrusible, and a cup-shaped ventral sucker situated a little behind the centre of the body. The margin of the ventral sucker is beset with minute tubercles and may protrude slightly.

Internal anatomy. A slightly dilated buccal cavity leads through a short pre-oesophagus to a spherical muscular pharynx. The latter is followed by a tubular oesophagus which bifurcates just anterior to the ventral sucker. The intestinal loops reach almost to the posterior extremity of the body.

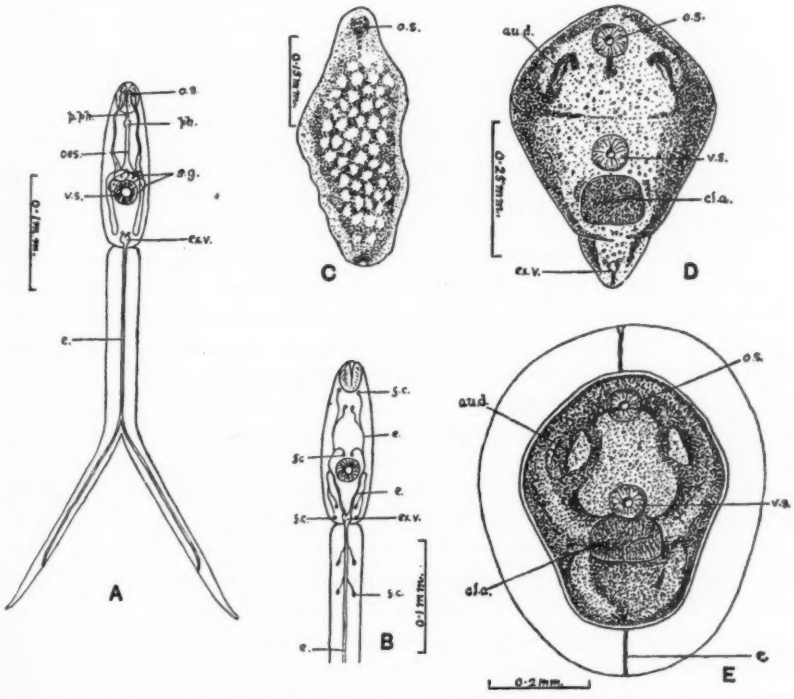

Fig. 1. Larval stages of *Strigea tarda* Steenstrup. A, Cercaria showing alimentary canal and glands. B, Cercaria showing excretory system. C–D, Precystic stages from *Limnaea peregra* Müll. E, *Tetracotyle typica* Dies. from *L. peregra* Müll.

au.d. auricular depression; *b.p.* birth pore; *cl.a.* "clasping apparatus"; *e.* excretory vessel; *e.s.* eye spot; *ex.v.* excretory vesicle; *f.c.* flame cell; *g.* rudiment of genital system; *int.* intestine; *oes.* oesophagus; *o.s.* oral sucker; *ph.* pharynx; *p.ph.* prepharynx; *s.g.* salivary gland cell; *v.s.* ventral sucker.

Four closely apposed salivary gland cells of diameter 0·01 mm. are situated immediately anterior to the ventral sucker. Their ducts are sinuous and dilate slightly in passing through the oral sucker.

The excretory vesicle is bluntly bilobed and receives two main collecting tubules which divide in the plane of the ventral sucker to form anterior and posterior lateral tubules. There is a distinct median caudal tubule dividing

near the bifurcation of the tail, each duct so formed opening on the margin of the fork about 0·06 mm. from the tip. There are seven flame cells on each side of the body, three connected with the anterior lateral tubule, two with the posterior lateral tubule and two with the median caudal tubule.

The genital system was not observed. Other measurements are given in Table I.

Table I.

	mm.
Oral sucker	0·03 × 0·02
Ventral sucker	0·02
Breadth of body in region of ventral sucker	0·04
Breadth of median caudal stem	0·03
Breadth of furci at base	0·019

Occurrence. On date of first finding, September 20th, 1924, the parasite occurred in one out of about twenty specimens of the host examined. During June and July 1925 it was present in approximately 10 per cent. of the *Limnaeas* examined.

Identification. There seems little doubt that this cercaria is identical with *Cercaria* A. Szidat (1924*a*, pp. 301–303) from the liver of *Limnaea palustris* Müll. My measurements are proportionately smaller than those given by that author, but the internal anatomy, especially as regards the number and arrangement of the salivary gland cells, is the same in both larvae. Further Szidat (*loc. cit.* pp. 304–13) has shown that *Cercaria* A. develops into *Tetracotyle typica* Dies. in the gonads of *Limnaea palustris* Müll. I have traced a similar development in *Limnaea peregra* Müll. for the cercaria described above.

Among the named European species the nearest relative is perhaps to be found in *Cercaria gracilis* La Val.

Development of Tetracotyle typica *Dies. in* Limnaea peregra *Müll.*
(Fig. 1 C–E.)

In order to continue their development the great majority of the cercariae migrate from their first host and actively seek a second, usually of the same species. The cercariae swim actively with the tail directed forwards and they are able to survive 24–30 hours in pond water. *Limnaeas* already infected with sporocysts, harbour as a rule few encysted stages. The transition from cercaria to encysted *Tetracotyle* occupies, under experimental conditions, from 25–30 days, during which time the larva alters its form considerably. During the summer and autumn of 1925 about 50 per cent. of young *Limnaeas* dissected were infected, as many as fifty larvae at various stages in development being common in one host. The usual number of completely encysted stages in one host however, is only from five to ten, so that of those cercariae actually entering only a small proportion are able to encyst.

Experimental. A. For experimental work *Limnaeas* were obtained from a locality where no helminth infection of this nature had been met with. About 150 young specimens of *L. peregra* Müll. were used and kept in pond

water in a small glass aquarium at room temperature. Cercariae were introduced on one occasion only when four infected digestive glands were teased into the water. The first of the *Limnaeas* were dissected approximately 17 hours after the introduction of the cercariae and thereafter at the rate of about five per day, several being fixed for sectioning. The experiment was performed during July and August, 1925.

I have not observed the actual penetration of the cercaria into the intermediate host, but the process is a rapid one. Szidat (1924*a*, p. 306) says that it seldom occupies more than half a minute, the cercariae having disappeared into the body of the host in 15–20 seconds. They penetrate usually in the region of the respiratory opening and continue to bore until they reach the reproductive organs. Several were found in this situation approximately 17 hours from the start of my experiment. Szidat does not state how long his larvae took to reach the hermaphrodite gland, but says that he found them there, and there only, several days after infection. I have found that, although the hermaphrodite gland is their chief habitat, the cercariae are also capable of undergoing a certain amount of development in the liver itself.

The first change in the cercaria is noticeable in the salivary gland cells which have almost completely disappeared by the time the hermaphrodite gland is reached. At a length of about 0·23 mm. the larva presents a flattened, oval, semi-transparent appearance with the characters of the cercaria rapidly disappearing. At 0·4 mm. there remains only a faint indication of the former ventral sucker, the rest of the larva presenting a curious net-work appearance of thin-walled vacuole-like cells, with small dark granular cells in the interspaces. As no trace of an intestine is visible at this phase in development feeding must be an endosmotic process, and it leads in most cases to the complete castration of the host. Growth in length ceases after the fifteenth day, and thereafter regeneration of the internal organs begins, the body contracting somewhat in the process. The oral and ventral suckers are first discernible. Later the auricular depressions in the anterior part of the body and the so-called "clasping apparatus" behind the ventral sucker are differentiated. Finally a cone-shaped portion becomes differentiated at the posterior end of the body. The whole then secretes a gelatinous membrane which gradually increases in thickness. Traversing this gelatinous outer zone, narrow excretory canals connect with the posterior and anterior ends of the larva.

The significance of the above-mentioned "clasping apparatus" situated behind the ventral sucker and appearing comparatively late in development, is not fully understood. Odhner (1913) in his work on the evolution of the Holostomes and the homologies of their suckers, points out its sucker-like appearance. Muhling (1896) says it resembles an ordinary sucker. The structure as seen in *Tetracotyle typica* Dies. is certainly sucker-like with a relatively long and narrow cleft in its centre, but with no marginal thickening. Faust (1918) traces in this organ a "primitive genital pore" leading to a vagina. Szidat found no such pore in *T. typica* Dies. and regards what might be mis-

taken for a vagina as simply a blind fold in the organ. From my own observations I have discerned no connection between the structure in question and the genital rudiments. The latter in my specimens show little or no differentiation. Szidat had difficulty in recognising two testes in his sections.

The size of the finished *Tetracotyle* varies slightly. Its average measurements are: Length 0·45 mm., breadth 0·38 mm. (excluding gelatinous wall), oral sucker 0·058 mm., ventral sucker 0·065 mm., "clasping apparatus" 0·09 mm. Table II contains a series of average measurements at various stages in the development of the cercaria into the *Tetracotyle*, together with remarks on some structural peculiarities to be seen as growth proceeds.

Table II.

Age	Measurements		Remarks
17 hours (approx.)	Length Breadth Oral and ventral suckers as in cercaria	0·165 mm. 0·049 mm.	Found in the hermaphrodite gland. Body spines and salivary glands indistinct
3–4 days	Length Breadth	0·23 mm. 0·08 mm. (max.)	Body spines and salivary glands disappeared. Ventral sucker, alimentary canal and excretory system indefinite. One sucker not traced
8–10 days	Length Breadth	0·46 mm. 0·16 mm. near centre of body tapering to 0·05 mm. at each end	Appearance as in Fig. 1 C
15–17 days	Length Breadth	0·66 mm. 0·3 mm. just anterior to centre of body	Regeneration of internal organs begins
20–22 days	Length Breadth Oral sucker Ventral sucker	0·55 mm. 0·35 mm. (max.) 0·055 mm. 0·06 mm.	Oral and ventral suckers fairly definite. Anterior auricular depressions developing
25 days	Length Breadth Oral sucker Ventral sucker "Clasping apparatus"	0·49 mm. 0·34 mm. (max.) 0·058 mm. 0·06 mm. 0·09 mm.	Secretion of wall begins. Appearance as in Fig. 1 D

The above development while agreeing in its essential points with that described by Szidat for *Cercaria* A. differs from it in the following respects. Szidat finds that the cercariae make for the reproductive organs of the host rapidly and does not find them in any other habitat several days after infection. From experiment and from observations on field material I find that although the hermaphrodite gland is the main seat of infection the cercariae are also capable of continuing the later stages of their development in the liver of the host. The latter habitat, however, appears to be unfavourable for their growth, and probably in part accounts for the fact that so many cercariae failing to reach the hermaphrodite gland first, perish before they attain to the encysted stage. Again, the time taken by *Cercaria* A. to complete its development in

Limnaea palustris Müll. is 20–25 days. In my own case in *L. peregra* Müll. it is 25–30 days, and even at the end of that time several mobile stages were met with.

Tetracotyle typica Dies. has been recorded from the following molluscs on the continent: *Limnaea stagnalis* L., *L. auricularia* L., *L. palustris* Müll., *Planorbis corneus* L., *Viviparus viviparus* L., and *V. fasciatus* Müll. Around Dundee and in North Fifeshire the chief host is *L. peregra* Müll., but I have also obtained it from *Physa fontinalis* L.

Development of Tetracotyle typica *Dies. into* Strigea tarda *Steenstrup.*

Experimental. B. During April, 1926, I conducted a number of controlled feeding experiments with *T. typica* Dies., using ducklings as final hosts. Cysts from *L. peregra* Müll. were first used and the adults obtained were identified by Dr Baylis as *Strigea tarda* Steenstrup. Infection occurs readily and development is normal. The parasite was located mainly in the first part of the small intestine. The period which elapsed between the time of infection and the appearance of eggs in the faeces was approximately 12 days. The experiment was repeated, using encysted stages from *Physa fontinalis* L., when a similar result was obtained.

The experiments of Ercolani (1881), Mathias (1924) and Szidat (1924 *a*, *b*) on the development of *T. typica* Dies. have already been referred to. Mathias in his preliminary communication describes very briefly the whole life cycle of this parasite. By experimental infection with miracidia and using *L. stagnalis* L. as intermediate host, he obtained sporocysts containing furcocercariae. The length of the individual sporocyst is given as 10–20 mm., which differs considerably from my own measurements of sporocysts from *L. peregra* Müll. Unfortunately Szidat gives no measurements of the sporocysts of *Cercaria* A. from *L. palustris* Müll. Mathias has not described the cercaria in detail and gives no measurements, so that comparison is difficult. The position of the salivary gland cells (he is not sure of their number) is, however, the same as in *Cercaria* A. and my own specimens. The same author finds that the development of the cercaria into *T. typica* Dies. in *L. stagnalis* L. occupies about 12 days, that is, roughly half the time taken by my own specimens or those of Szidat. Further, in his feeding experiments he found eggs in the faeces of the duck only $3\frac{1}{2}$ days after infection as compared with 12 days in my own experiments.

It might be added that Vevers (1923) fed a number of encysted larval Trematodes which he believed to be *T. typica* Dies., to ducks, with negative results. He concluded that this larva, which he obtained from *L. peregra* Müll., does not develop in the domestic duck, or if it does, it produces adults so small as to be easily passed over at a post-mortem examination. While the conditions obtaining in the experiments of that author may have been unfavourable for the development of *T. typica* Dies. it seems more probable that he was not dealing with this larva at all or with immature specimens of the species.

Cercaria F.1 (Fig. 2 A–E).

The Sporocysts were found in the liver and gonads of *Planorbis crista* L. and of *P. albus* Müll. They are thin walled and translucent. Mature specimens vary in length from 2–3·5 mm. The diameter, 0·056 mm., is fairly uniform, and the wall which is composed of a single layer of cells is approximately 0·009 mm.

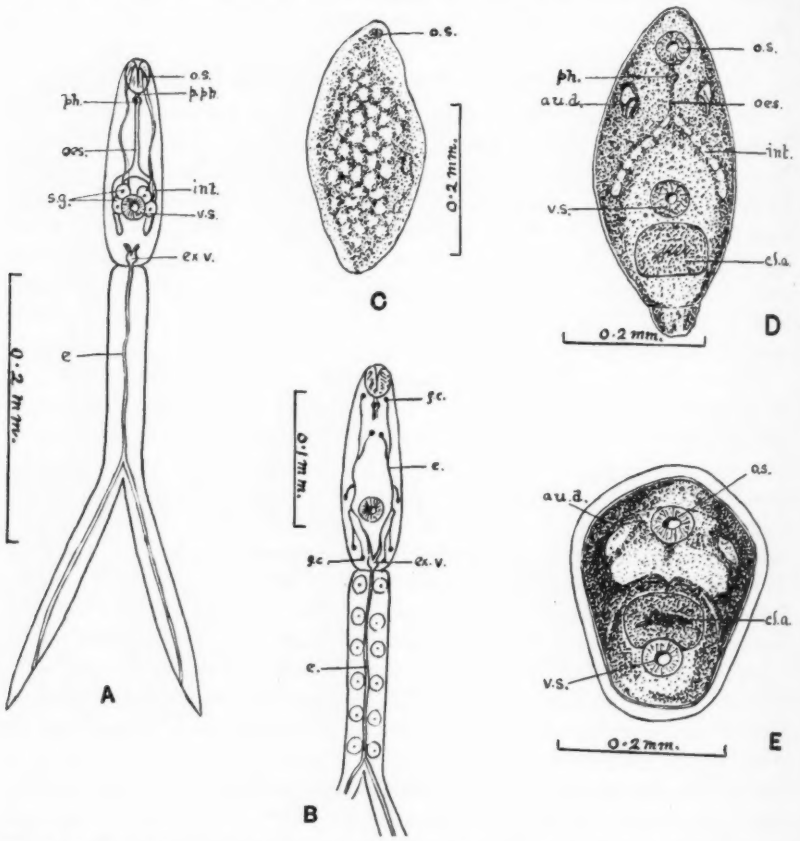

Fig. 2. *Cercaria* F.1. A, Cercaria showing alimentary canal and glands. B, Cercaria showing excretory system. C–D, Precystic stages from *Planorbis crista* L. E, Encysted *Tetracotyle* from *P. crista* L.

(Key to lettering under Fig. 1, p. 313)

thick. At maturity, the greater part of the body of the sporocyst projects from the surface of the liver and exhibits peristaltic-like movements of its walls, in addition to the usual side to side movement. The motion is visible through the shell in infected specimens of *P. crista* L., the smaller of the two hosts. The area of cell proliferation is at the extreme anterior end of the

sporocyst, and at an early stage in their development the cercariae become separated from the germinal area and are kept in continual movement backwards and forwards in the lumen, by the movements of the parent larva. A birth pore is present in mature specimens about 0·08 mm. from the anterior end.

The Cercaria is translucent and exceedingly contractile. The body and median shank of the tail are of about equal length (0·14 mm.), while the flattened furci measure 0·18 mm. The anterior third of the body is covered with minute spines which extend into the oral cavity. The oral sucker is ovate and its inner walls are protrusible. The ventral sucker is situated a little posterior to the centre of the body and is armed on its margin with an inner row of small tubercles and an outer row of short bristles.

Internal anatomy. A small spherical pharynx lies just behind the oral sucker. The oesophagus bifurcates about the centre of the body, and the loops of the intestine reach a little below the level of the ventral sucker.

Two pairs of salivary gland cells lie on either side of the ventral sucker. Their ducts pass anteriorly to open into the oral cavity.

The excretory vesicle is small and bluntly bilobed. Anterior and posterior lateral tubules unite on either side of the body about the level of the ventral sucker to form a main collecting tubule leading into the arm of the vesicle. Posteriorly, the vesicle connects with a median canal in the tail. Six flame cells were observed on each side of the body, three connected with the anterior lateral tubule, two with the posterior lateral tubule, and one in the tail. The connection of the caudal flame cells with the system was not traced. The ducts formed by the bifurcation of the median caudal duct open on the inner margins of the forks near the tips.

Six larger cells of diameter 0·013 mm. lie on either side of the main caudal vessel. These cells are clear and thin walled and contain prominent nuclei. Cells, probably of a similar nature, occur in the tail of a furcocercous larva (unnamed) described by Hesse (1923) from *Limnaea peregra* Müll., and who has suggested an excretory function for them. Brown (1926) also records six pairs of large well-defined cells apparently attached to the excretory duct, in the tail of *Cercaria micromorpha* n.sp. from *Limnaea peregra* Müll.

Other measurements are given in Table III.

Table III.

	mm.
Oral sucker	0·025 × 0·02
Ventral sucker	0·025
Breadth of body in region of ventral sucker	0·032
Breadth of median caudal stem	0·028
Breadth of furci at base	0·013

Identification. *Cercaria aculeata* Erc., and *Cercaria letifera* n.sp. (Fuhrmann, 1916), both from *Limnaea auricularia* L., are the only species comparable. The sporocysts of *C. letifera* n.sp. have approximately the same length as

in the species described here but show no birth pore, while the sporocysts of *C. aculeata* Erc. although possessing a birth pore measure only 1·0 mm. long by 0·08 mm. in greatest thickness. In *C. aculeata* Erc. the ventral sucker is situated as in my own species but is unarmed; in *C. letifera* n.sp. it is in the anterior third of the body. Again in *C. letifera* n.sp. the median stem of the tail bears numerous tactile bristles and has regular annulations on its surface. Finally in their behaviour to secondary intermediate hosts *C.* F.1 and *C. letifera* n.sp. are quite different.

Development of C. *F.*1 *into* Tetracotyle *n.sp. in* Planorbis crista *L. and* P. albus *Müll.* (Fig. 2 C–E).

The development of *C.* F.1 into the encysted *Tetracotyle* is essentially the same as the development of *T. typica* Dies. The hermaphrodite gland of the mollusc is again the main seat of infection for mobile precystic stages. The encysted stage in addition to being found in the hermaphrodite gland occurs also, but less commonly in the liver. During October, 1924, over 90 per cent. of *Planorbis crista* L. and from 8–10 per cent. of *P. albus* Müll. from Morton Loch, Fifeshire, were infected with sporocysts, many hundreds of specimens of each species being dissected. During the autumn of 1925, developmental stages of the *Tetracotyle* were found in the gonads and liver of almost every specimen of *P. crista* L., and in about one-tenth the number of *P. albus* Müll. examined from the same locality. While many free larval stages were common in one host it was rare to find more than twelve completely encysted stages in one mollusc. The duration of free life of the cercaria is short, being only from 6 to 10 hours in pond water.

Experimental. A. Some difficulty was at first experienced in obtaining specimens of *P. crista* free from infection of this nature, but these were eventually procured from a small loch near Perth. The experiment was conducted on similar lines to that described for *T. typica* Dies. (p. 317). The characters of the cercaria again disappear early when the larva assumes a flattened semi-transparent network appearance. Growth in length and breadth is rapid and ceases about the twelfth day, when regeneration of the internal organs accompanied by some contraction of the body begins. The auricular depressions on either side of the oral sucker are short and unlike those of *T. typica* Dies., possess at their anterior ends a clear lobe-like structure which projects some distance from the body. Just before encystment which begins about the eighteenth day the intestine can be made out. It consists of a small pharynx a short distance behind the oral sucker, a short oesophagus, and a triclade intestine, the loops of which reach to the level of the ventral sucker. At this stage the intestinal loops are composed of a single row of cells arranged end to end. As encystment proceeds the posterior end of the larva becomes bent ventrally so that the "clasping apparatus" comes to lie above, and in some cases anterior to the ventral sucker. The final encysted stage is greyish white

in colour and bluntly rounded at both ends, the maximum breadth being anteriorly.

A similar experiment was performed using *P. albus* Müll. as host.

Table IV contains a series of average measurements at different stages in the development of the cercaria into the encysted stage.

Table IV.

Age about 5 days	Length	0·22 mm.
	Breadth	0·13 mm.
Age about 12 days (Fig. 2 C)	Length	0·34 mm.
	Breadth	0·16 mm.
Age about 18 days	Length	0·44 mm.
	Breadth	0·2 mm.
	Oral sucker	0·04 mm.
	Ventral sucker	0·06 mm.
	"Clasping apparatus"	0·12 mm.
Final encysted stage Age 20 days	Length	0·3 mm.
	Breadth anteriorly	0·22 mm.
	Breadth posteriorly	0·19 mm.
	Suckers as above	

B. During the summer 1926 I repeatedly fed the *Tetracotyle* described above to ducklings. Post-mortem examination of the whole intestine and its associated ducts, however, failed to reveal a corresponding adult.

Cercaria F.2 (Fig. 3 A–C).

The Sporocysts occur in the liver and gonads of *Planorbis albus* Müll., and when mature are thread-like and very involved. The length is 3·5–4 mm. and the breadth about 0·07 mm. When alive they are greyish white and often tinged with a yellowish brown pigment at their free anterior ends. Transverse constrictions are numerous. A birth pore occurs about 0·08 mm. from the anterior end.

The Cercariae is translucent with a stout almost cylindrical body 0·18–0·2 mm. long. The median stem of the tail measures 0·25 mm. long and bears two short lanceolate forks 0·14 mm. long at its distal end. Standing out in sharp contrast to the hyaline body of the living larva, are two black pigment eye spots situated superficially on the anterior dorsal aspect of the body. The anterior third of the larva has a sparse covering of very minute spines. The oral sucker is indistinct and its walls are not protrusible. The ventral sucker, on the other hand, is well developed and is located a little behind the centre of the body. It is armed with small tubercles on its outer margin. The median caudal stem is exceedingly contractile and exhibits muscular longitudinal and transverse striations. The furci are slightly constricted from the median stem, and project from it almost at right angles when the larva is at rest.

Internal anatomy. The oral cavity leads directly to a tubular oesophagus which bifurcates a short distance below the level of the pigment eye spots. The loops of the intestine reach to the level of the anterior margin of the ventral sucker. A pharynx is apparently lacking.

Three finely granular salivary gland cells are arranged on either side of, and above the ventral sucker. They are dorsal in position and communicate by means of two ducts with the oral cavity.

The genital system is represented by a single pear-shaped mass of small cells situated ventrally about mid-way between the ventral sucker and the posterior margin of the body.

The excretory vesicle is small and spherical. Two main collecting tubules lead from it anteriorly to almost the level of the ventral sucker where they

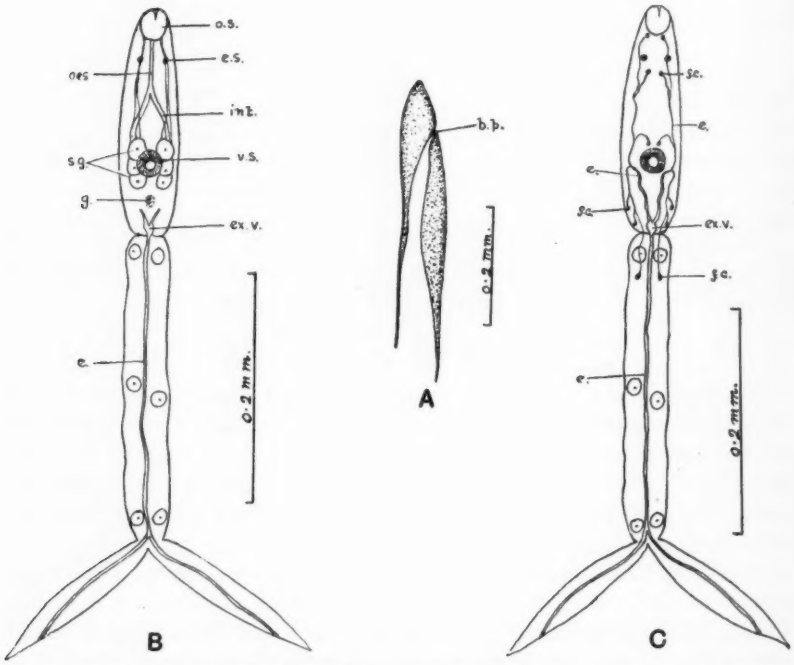

Fig. 3. *Cercaria* F.2. A, Anterior end of sporocyst showing birth pore. B, Cercaria showing alimentary canal and glands. C, Cercaria showing excretory system.
(Key to lettering under Fig. 1, p. 313)

form a slight bay near the sides of the body. Here, each receives an anterior and a posterior lateral tubule. The median portions of the primary collecting tubules are ciliated. The anterior lateral tubule on each side receives three capillaries with flame cells, one near the margin of the oral sucker, another just posterior to the eye spots, and the third at the anterior edge of the ventral sucker. Each posterior lateral tubule is also associated with three flame cells, two near the posterior end of the body, and a third in the proximal portion of the caudal stem. A fine median duct passes from the posterior end of the vesicle into the tail. This duct bifurcates at the fork, each tributary so formed opening

on the inner margin of the fork near its tip. Three large cells occur on each side of the median caudal duct, and these may also have an excretory function.

Other measurements are given in Table V.

Table V.

	mm.
Oral sucker	0·02
Ventral sucker	0·014
Breadth of body at level of ventral sucker	0·045
Breadth of median caudal stem	0·03
Breadth of furci at base	0·014
Diameter of eye spots	0·003
Distance of eye spots from anterior end...	0·04

Cercaria F.2 occurred in about 5 per cent. of the specimens of *P. albus* Müll. examined during July and August, 1925.

The duration of free life is from 15 to 20 hours in pond water.

Identification. *Cercaria ocellata* La Val., and *C. macrosoma* n.sp. (Brown, 1926), both from *Limnaea stagnalis* L., are the only European species of oculate furcocercariae comparable. The relative sizes of its suckers, and the frilled margins of the tail furci, in *C. ocellata* La Val. mark it as distinct from *C.* F.2, while *C. macrosoma* n.sp. differs in having its whole body armed with spines, in the position of its salivary gland cells, and in possessing a pharynx.

IV. SUMMARY.

Three species of furcocercariae are described. By means of direct animal experiment with secondary, intermediate and final hosts I have shown that one species develops into *Tetracotyle typica* Dies., the larva of *Strigea tarda* Steenstrup, and a connected detailed account of this life cycle is here given. Of the two other species, both of which are new, I have been able to trace the development of one into *Tetracotyle* n.sp. in *Planorbis crista* L., and *P. albus* Müll. Feeding experiments with this new Tetracotyle using ducklings as final hosts failed, however, to produce the corresponding adult.

Effect of the parasite on the host. The usual results of helminth infection of this nature are observable, namely, marked disruption of liver tissue and in many cases complete parasitic castration of the molluscs infected.

Double infection. In the course of my investigations on larval Trematodes I have met with one example of double infection with sporocysts, viz. *Cercaria* F.1 and F.2, within one and the same individual of *Planorbis albus* Müll. This condition was found only on one occasion, *Cercaria* F.1 being in predominance. Instances of double infection have been recorded by Faust (1917) and Sewell (1922), and from this country by Hesse (1923) and Brown (1926). The condition, however, does not appear to be a common one.

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Strigea tarda Steenstrup, and to Mr H. Coates, F.S.A. (Scot.), of Perth, for the names of the Molluscan hosts.

I am also indebted to Prof. D. Rutherford Dow, M.D., in whose department this paper was prepared.

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TRYPANOSOMA GAMBIENSE IN MONKEYS AND RUMINANTS; PROLONGED INFECTION, IMMUNITY AND SUPERINFECTION.

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In the *Final Report of the League of Nations International Commission on Sleeping Sickness* (1928) certain tentative conclusions were expressed about the behaviour of *T. gambiense*, recently isolated from man, in the blood of monkeys and of certain domestic ruminants. These conclusions, which were intended primarily to serve as a basis for further researches, were set forth in the Summary of Part I of that Report, and read as follows:

"1. That, as a general rule, the transmissibility of a strain of *T. gambiense* diminishes when the strain is introduced into a sheep or goat.

"2. That after some months' sojourn in these animals, *T. gambiense* loses its transmissibility altogether.

"3. That a strain of *T. gambiense* may lose its transmissibility quite suddenly on being transferred from one host to another by cyclically infected *G. palpalis*.

"4. That, in the three patients whose strains were transmitted by tsetse fed directly on the patient, the trypanosome had a higher Transmissibility Index in man himself than in the animals infected from him by cyclical transmission.

"5. That it is improbable that sheep and goats play any important part as a reservoir of this trypanosome. According to these experiments, calves are a negligible factor in this respect."

In a later publication (Duke, 1928*a*) an account was given of the extension of these studies to the subsequent history of some of the animals dealt with in the Report, including the results of superinfection with either homologous or heterologous strains of *T. gambiense*, or both in turn. The particular manifestation of the existence of immunity in the chronically infected animals of these later experiments was the effect produced by the mammal upon the transmissibility of its trypanosomes by *Glossina palpalis*. The method pursued was, briefly, the employment at intervals of batches of several hundred clean laboratory-bred tsetse, each batch constituting a "test," to determine the extent to which the trypanosome had maintained its transmissibility by tsetse. In each case superinfection was carried out by feeding on the animal flies proved to be infective with the particular strain of *T. gambiense* which it was

desired to introduce. These later studies led to some interesting observations, and indicated the desirability of continuing the work on similar lines.

Briefly stated the main inferences drawn from these later experiments were as follows:

(1) That natural resistance against *T. gambiense*, exhibited by sheep and goats in Uganda, varies from complete immunity to a degree of susceptibility in which the trypanosome may be a contributory, or even possibly the direct cause of death, this last contingency being undoubtedly exceedingly rare. That there exists also a peculiar partial immunity which operates on the transmissibility of the trypanosome by tsetse, abolishing the power to invade the salivary glands, but leaving the strain still capable of infecting the gut of the fly and still demonstrable by ordinary means in the peripheral blood of the mammal.

(2) That immunity against *T. gambiense* can be acquired by sheep and goats, the earliest manifestation of such immunity being the loss of power to invade the glands of tsetse. The power of the trypanosome to develop in the fly's gut may persist for some time, or this may also disappear more or less rapidly. Superinfection of such a partially immunised animal with a transmissible strain of *T. gambiense* failed to render the blood of the animal again infective to the glands of tsetse.

(3) In contrast to the above sequence of events, in certain animals a condition of equilibrium was established between mammal and trypanosome, the strain remaining infective to the glands of the fly for a considerable period, a state of affairs similar to that already described in certain antelope experimentally infected with *T. gambiense* (Duke, 1912).

In the case of one of the two monkeys studied the transmissibility of the original strain disappeared; subsequent infection of the animal with a homologous strain then temporarily restored the transmissibility; and, finally, superinfection with a heterologous strain of *T. gambiense* again rendered the blood of this monkey readily infective to tsetse.

Between the conclusions reached in these two reports there is no essential disagreement. The most striking divergence is the record in the later paper of survival for 15 months in Sheep 161 of *T. gambiense* in a form transmissible by tsetse, an observation that rendered necessary the collection of further evidence bearing on conclusions 2 and 5 of the Commission Report.

In the studies now to be described the history of several of the animals described in 1928 is completed up to their death, in one case only a few months ago.

For the sake of continuity and clarity tabular summaries of the previously published tests with each animal are given first in chronological order, and then subsequent and hitherto unpublished examinations.

It is neither desirable nor possible here to deal with all the points raised in the two previous publications. The peculiar partial immunity, for example, in face of which a strain loses its gland-infecting powers, is left for the time being

untouched. The object of the present paper is to submit further evidence on two important items of the Research Programme of the Institute; first, the ability of ruminants, domestic and otherwise, to serve as reservoirs of *T. gambiense*, and secondly, the effect on this species of trypanosome of prolonged sojourn in a single mammalian host.

In addition to the extended examination of some of the animals, an account of whose superinfection has already been published, there is presented here all the evidence now available on these two subjects in the Entebbe records, old and new, published and unpublished.

Every instance where an infected animal, monkey or ruminant, has been subjected to more than one "test" with clean laboratory-bred flies is given impartially below, so that all the main observations upon which rest the theories I have advanced on these two topics are now laid upon the table in the two papers referred to above and in a publication in *Parasitology* (Duke, 1930).

It is not claimed that a final settlement of these questions has been reached. Conclusions in work of this kind crystallise slowly and many "tests" prove sterile. Moreover the limits and exigencies of normal life in these animals at the laboratory make repeated testing difficult.

In such circumstances it is gratifying to secure any sort of coherence in experimental results, and it is hoped that the fresh evidence submitted in this paper will help to solve some of the problems that still obscure our understanding of the biology of *T. gambiense* and its allies.

The method of recording the experimental data is that described and used in the Commission Report. A few comments on this subject are expedient here to help towards a rapid appreciation of the salient points in the experimental records that follow.

The Transmissibility Index of a strain is calculated from the following formula:

$$T = \frac{s}{p} \times \frac{n \times 100}{N},$$

where N = total number of flies dissected, n = total number of flagellate-containing flies; p = number of infected flies twenty-five days old or over, and s = the number of these mature infected flies that shows gland infections.

The information afforded by each fly test is summarised in a table comprising nine vertical columns which in the specimen given below are for convenience numbered 1–9.

					No. of infected flies alive at 25th day			
Test	Date	Total flies dissected	Total infected flies	% of infected flies	With glands infected	Total	% of gland infections	Trans-missibility index
1	2	3	4	5	6	7	8	9

In interpreting such a summary the important columns are Nos. 5, 6 and 7. No. 5 shows the total percentage of infected flies of all those dissected during

the test. This number is valuable both as an indicator of the adjustment of the strain of tsetse, and in comparing different strains or different phases of the same strain with one another. This number, known as the *Infectivity Index*, is not subject to the accidents that may affect columns 6 and 7. Columns 6 and 7 relate only to infected flies that have lived twenty-five days or longer, *i.e.* sufficient time for the salivary glands to become infected if infection is going to occur. Long experience has convinced me that, at all events with *G. palpalis*, a fly whose glands are not infected by the twenty-fifth day after the last infecting feed will never become infective. Infection of the glands often occurs earlier than this, and Robertson (1912) records an instance of infection of the glands as early as the twelfth day of the experiment. In my own experience the sixteenth day is the earliest date on which a gland infection has been observed.

The first point to note about columns 6 and 7 is the degree of correspondence between column 7 and column 4. When these two are identical, that is when every infected fly lives long enough to develop gland infection, then the test may be regarded as truly representative. The greater the difference between these two columns the less the value of the test in appraising the transmissibility of the strain. When the metacyclic index $\left(\frac{s}{p}\right)$ is unity the infectivity and transmissibility indices are the same. It follows also that the infectivity index represents the highest potential transmissibility of the strain at the time of the test, subject of course to the limitations of the method of experiment.

It has been shown, it is hoped conclusively, in earlier work from this laboratory that a strain of trypanosomes originally isolated from wild tsetse or from naturally infected mammals, may in the course of prolonged upkeep by syringe passage at the laboratory lose its transmissibility by tsetse (Duke, 1924). The change is signalised first by the loss of the power to invade the glands and later, sometimes long later, by the failure to infect even the gut of the fly. We are now concerned in following the changes in transmissibility in strains kept under conditions more nearly approaching those obtaining in nature, by employing cyclically infected tsetse instead of the syringe and by studying the strain at intervals during its career in one and the same host.

In addition to their bearing on the main objectives of this paper the tests set forth below illustrate the use and the utility of a new method of studying trypanosomes, *i.e.* the employment of clean tsetse. This method has been both fairly criticised and, in my opinion, hastily condemned. I would ask the reader to approach this review with an open mind and to decide on the evidence before him whether or not the degree of consistency exhibited by the results warrants the conclusions drawn, and at the same time ratifies the claims, explicit and implied, made for the method.

The summaries of the fly tests performed with various animals infected with *T. gambiense* will now be given.

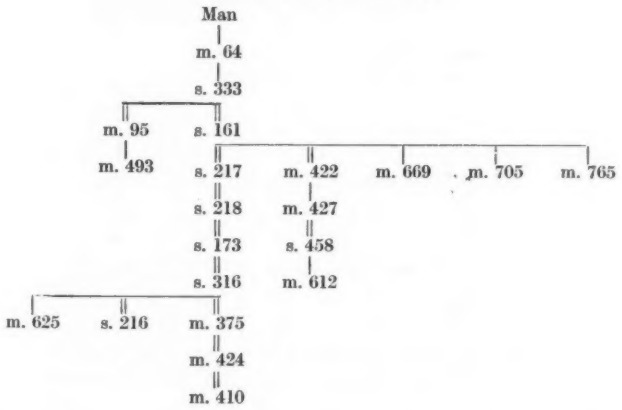
A "test" consists of a collection of fly experiments each of which is a box

containing at the commencement fifty laboratory-bred *G. palpalis*. The period covered by a test is shown in the date column. The first date refers to the first feed of the first experiment, the second date to the last feed of the last experiment of the test. Each experiment is fed upon the infected animal usually on two alternate days with a day's starvation in between and the boxes are then nourished on a clean animal or fowl until the final dissection. As a general rule one experiment feeds on the infecting animal each day during the period of the test, though sometimes there are days within that period on which no flies are put on.

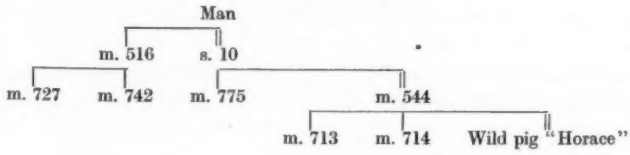
All the summaries marked with an asterisk have been already published; the others are new.

The investigations now to be set forth embrace six different strains of *T. gambiense*, but the great majority relate to Strains V and XXXIII, both of which had been selected for prolonged maintenance by cyclical passage at the laboratory. The history of these strains since they arrived at the laboratory is given in the following genealogies, where a single line means syringe passage, a double line cyclical passage through *G. palpalis*; m. signifies monkey, s. sheep, g. goat. In every case where superinfection was practised the animal was fed upon by flies that were proved infective to clean animals immediately before and immediately after the superinfecting feed. Animals exposed to superinfection will be taken first.

Strain V (T. gambiense).



Strain XXXIII (T. gambiense).



Strain XXVII (T. gambiense).

Man
|
m. 407
||
m. 442
||
situtunga

Strain XXIX (T. gambiense).

Man
|
m. 507
|
m. 506
|
m. 607

Strain II (T. gambiense).

Man
|
m. 53
|
s. 332

Strain VI (T. gambiense).

Man
|
m. 65
|
g. 341

Strain XLVIII (Lakeshore *strain*).

Wild fly
||
dog
|
m. 387
||
s. 300

Strain XXVIII (T. gambiense).

Man
|
m. 487

STRAIN V (*T. GAMBIENSE*).

This strain was sent to Entebbe from Kavirondo by Prof. Kleine in m. 64 which was inoculated from a native on 16. iii. 26.

Monkey 95: infected by flies on 23. v. 26: died 2. xii. 28.

Test	Date	Total flies dissected	Total infected flies	% of infected flies	No. of infected flies alive at 25th day: With glands infected	No. of infected flies alive at 25th day: Total	% of gland infections	Transmissibility index
*1	x. 26	89	15	16·8	2	5	2·2	*6·7*
*2	ii. 27	77	3	3·8	3	3	3·8	*3·8*
*3	v–vi. 27	228	5	2·1	0	4	0	*0*
	(Superinfected with Strain V on 13 and 14. vii. 27.)							
*4	vii–viii. 27	376	7	1·8	3	5	0·7	*1·0*
*5	viii–ix. 27	324	0	0	0	0	0	*0*
*6	xi–xii. 27	181	4	2·2	0	1	0	*0*
	(Superinfected with Strain XXVII on 17 and 19. xii. 27.)							
*7	xii. 27–i. 28	335	14	4·1	7	11	2·0	*2·6*
8	iv. 28	476	5	1·0	2	4	0·4	*0·5*
9	x. 28	552	15	2·7	4	13	0·7	*0·8*
	(Blood from m. 95 subinoculated into m. 493 on 22. xi. 28.)							
m. 493	i. 29	205	10	4·8	3	6	1·4	*2·4*

Each infection, primary or secondary, is thus characterised by a gradual diminution in the transmissibility by fly of the trypanosomes in this monkey. In the sixth test only one out of four infected flies lived twenty-five days. It should be noted that the strain in m. 493, released we may suppose from the restraining influences of 95's tissues, regained considerably its transmissibility by tsetse. This phenomenon will receive attention later on in this paper (cf. Strain XXXIII, monkeys 544, 516, etc.).

Monkey 375: infected by flies 18. vi. 27: died 20. v. 28.

Test	Date	Total flies dissected	Total infected flies	% of infected flies	No. of infected flies alive at 25th day: With glands infected	No. of infected flies alive at 25th day: Total	% of gland infections	Trans-missibility index
*1	18–27. vii. 27	253	8	3·1	3	6	1·1	*1·5*
*2	18–27. xi. 27	106	1	0·5	1	1	0·5	*0·5*
	(Superinfected with Strain XXVII on 11. xii. 27.)							
*3	xii. 27–i. 28	265	11	4·1	4	10	1·4	*1·6*

Sheep 333: infected 20. iv. 26 by the syringe: died 19. ix. 28.

Test	Date	Total flies dissected	Total infected flies	% of infected flies	No. of infected flies alive at 25th day: With glands infected	No. of infected flies alive at 25th day: Total	% of gland infections	Trans-missibility index
*1	7–20. vii. 26	150	9	6·0	5	7	3·3	*4·2*
*2	10. i–10. ii. 27	252	2	0·7	0	2	0	*0*
*3	22. vii–8. viii. 27	300	0	0	0	0	0	*0*
	(Superinfected with Strain V on 19. viii. 27.)							
*4	30. viii–28. ix. 27	553	3	0·5	0	3	0	*0*
	(Superinfected with Strain XXVII on 15. xii. 27.)							
*5	27. xii. 27–18. i. 28	290	0	0	0	0	0	*0*

Test 4 is a good one. Superinfection with the same and then four months later with a different strain of *T. gambiense* produced no apparent effect.

Sheep 161: original infection 24. viii. 26 by flies: died 20. i. 31.

Test	Date	Total flies dissected	Total infected flies	% of infected flies	No. of infected flies alive at 25th day: With glands infected	No. of infected flies alive at 25th day: Total	% of gland infections	Trans-missibility index
*1	30. ix–14. x. 26	252	27	10·1	12	22	5·1	*5·5*
*2	31. i–9. ii. 27	224	3	1·3	0	1	0	*0*
*3	15. xi–25. xi. 27	192	3	1·5	1	3	0·4	*0·5*
	(Superinfected with Strain XXVII on 11. xii. 27.)							
*4	22. xii. 27–16. i. 28	236	4	1·6	0	1	0	*0*
5	5. vi–18. vi. 28	494	3	0·6	0	3	0	*0*
6	5. ix–26. ix. 29	439	1	0·2	0	0	0	*0*
7	27. xi–11. xii. 29	353	1	0·2	0	0	0	*0*
8	24. v–25. vi. 30	436	0	0	0	0	0	*0*
9	5. viii–30. viii. 30	564	0	0	0	0	0	*0*
10	25. x–11. xi. 30	605	1	0·1	0	0	0	*0*
	(Subinoculations of the blood of sheep 161 into clean monkeys.)							
On 7. ii. 30 m. 669.	18. ii–19. iii. 30	612	0	0	0	0	0	*0*
On 24. vi. 30 m. 705.	15. vii–11. viii. 30	494	1	0·2	0	0	0	*0*
On 9. x. 30 m. 765.	25. x–17. xi. 30	548	9	1·6	0	7	0	*0*

At the date when superinfection of this sheep was performed the original strain in the animal was still capable of infecting the glands of tsetse, *i.e.* some fifteen and a half months after the original infection.

After superinfection no more gland infections were obtained in the 3127 flies employed, nor did the infectivity index of the strain increase.

In contrast to the behaviour of m. 95 three successful subinoculations of s. 161's blood into monkeys failed to reveal any revival of transmissibility, the strain having apparently permanently and completely lost this property during its sojourn in the sheep. In the test applied to m. 765 seven out of the nine infected flies lived twenty-five days, but none had gland infections. These subinoculations suggest that some hosts are more favourable to the trypanosome than others.

Monkey 64: infected 16. iii. 26 by the syringe from man: died 20. x. 26.

Test	Date	Total flies dissected	Total infected flies	% of infected flies	No. of infected flies alive at 25th day: With glands infected	No. of infected flies alive at 25th day: Total	% of gland infections	Transmissibility index
*1	20. iv–7. v. 26	154	8	5·0	7	7	4·5	*5·0*
*2	7–16. x. 26	179	16	8·8	2	9	1·1	*1·9*

Sheep 217: infected by flies on 25. x. 26: died 22. xii. 27.

Test	Date	Total flies dissected	Total infected flies	% of infected flies	No. of infected flies alive at 25th day: With glands infected	No. of infected flies alive at 25th day: Total	% of gland infections	Transmissibility index
*1	19. xi–11. xii. 26	224	12	5·3	3	5	1·3	*3·1*
*2	9–22. iii. 27	110	0	0	0	0	0	*0*

Sheep 218: infected by flies on 21. xii. 26: died 27. iv. 29.

Test	Date	Total flies dissected	Total infected flies	% of infected flies	No. of infected flies alive at 25th day: With glands infected	No. of infected flies alive at 25th day: Total	% of gland infections	Transmissibility index
*1	4–19. i. 27	225	2	0·8	1	2	0·4	*0·4*
*2	16. ii–11. iii. 27	152	5	3·8	3	4	1·9	*2·8*
3	22. i–11. ii. 28	311	5	1·6	2	2	0·6	*1·6*
4	29. vi–9. vii. 28	429	7	1·6	1	7	0·2	*0·2*
5	2. ii–21. iii. 29	273	1	0·3	0	0	0	*0*

The strain in this animal was thus still transmissible by tsetse eighteen months after its original infection by fly.

In the second test the transmissibility is considerably greater than that in the first. The probable explanation of this, strongly supported by experience with a number of animals in similar circumstances, lies in the fact that a certain period must elapse after the actual infection of an animal before the strain settles down in its new environment. Reference to the account of Strain V in the Commission Report shows that in the experiments of the first test no infected flies were found save only in the last box which fed on January 16th, 18th and 19th. By the time the second test was applied the strain was comfortably established and had not yet begun to feel the influence of the tissue reactions of the host.

The existence of this latent period must be borne in mind, especially in experiments with superinfection. If the clean flies of the test are applied to the animal too soon after superinfection, experiments have shown that misleading results may be obtained.

Sheep 173: infected by flies on 10. i. 27: died 21. vii. 29.

					No. of infected flies alive at 25th day			
Test	Date	Total flies dissected	Total infected flies	% of infected flies	With glands infected	Total	% of gland infections	Transmissibility index
*1	28. ii–29. iii. 27	151	5	3·3	4	4	2·6	*3·3*
2	22. i–13. ii. 28	323	4	1·2	2	4	0·6	*0·6*

A year after its introduction into this sheep the strain was still transmissible by tsetse.

Sheep 316: infected by flies on 18. iv. 27: died 14. xi. 29.

					No. of infected flies alive at 25th day			
Test	Date	Total flies dissected	Total infected flies	% of infected flies	With glands infected	Total	% of gland infections	Transmissibility index
*	20. v–16. vi. 27	321	8	2·4	2	5	0·6	*0·9*

On 13. xi. 29, blood of this sheep was inoculated into clean m. 625.

Monkey 625:

					No. of infected flies alive at 25th day			
Test	Date	Total flies dissected	Total infected flies	% of infected flies	With glands infected	Total	% of gland infections	Transmissibility index
1	12–30. xii. 29	381	8	2·1	5	7	1·3	*1·7*

The strain was thus transmissible by tsetse some two years and eight months after the original infection of the sheep.

Sheep 216: infected by flies on 2. vii. 27: died 29. xi. 28.

					No. of infected flies alive at 25th day			
Test	Date	Total flies dissected	Total infected flies	% of infected flies	With glands infected	Total	% of gland infections	Transmissibility index
1	14. vii–8. viii. 27	453	7	1·5	0	1	0	*0*
2	16–29. x. 28	310	2	0·6	1	2	0·3	*0·3*

The strain was still transmissible eighteen months after the original infection of the sheep.

Here again the transmissibility is greater in the later than in the earlier test. The obvious explanation is that only one out of the seven infected flies lived long enough for trypanosomes to reach the salivary glands and the first test is therefore valueless as an indication of transmissibility, the infectivity index is unaffected by this accident.

Monkey 422: infected by flies on 24. xii. 27: died 13. x. 29.

Test	Date	Total flies dissected	Total infected flies	% of infected flies	No. of infected flies alive at 25th day: With glands infected	No. of infected flies alive at 25th day: Total	% of gland infections	Transmissibility index
1	7–19. i. 28	174	7	4·0	1	2	0·5	*2*
2	20. ii–18. iii. 29	295	4	1·3	1	2	0·3	*0·6*
3	10. ix–4. x. 29	511	7	1·3	2	5	0·3	*0·5*

This monkey was a big strong animal still in good condition when it died as the result of an accident. Its trypanosomes were still transmissible two years and four weeks after the original infection. The first test gives a poor indication of the transmissibility of the strain, as only two out of seven infected flies lived twenty-five days.

Monkey 427: infected on 6. i. 28 by the syringe: died 14. xii. 29.

Test	Date	Total flies dissected	Total infected flies	% of infected flies	No. of infected flies alive at 25th day: With glands infected	No. of infected flies alive at 25th day: Total	% of gland infections	Transmissibility index
1	22–29. vi. 28	311	6	1·9	3	6	0·9	*0·9*
2	11. viii–2. ix. 28	303	6	1·9	4	4	1·3	*1·9*
3	22. ii–21. iii. 29	313	4	1·2	0	1	0	*0*
4	26. v–28. vi. 29	412	2	0·4	1	2	0·2	*0·2*

The strain was transmissible after eighteen months' sojourn in the monkey. The third test, it will be seen, is unsatisfactory because only one out of four infected flies lived twenty-five days, but the infectivity index has not altered much.

Sheep 458: infected by flies on 27. vii. 28: died 5. x. 29. No test was performed with this sheep, but on 5. x. 29 its blood was inoculated into clean m. 612.

Monkey 612:

Test	Date	Total flies dissected	Total infected flies	% of infected flies	No. of infected flies alive at 25th day: With glands infected	No. of infected flies alive at 25th day: Total	% of gland infections	Transmissibility index
1	17. x–19. xi. 29	398	4	1·0	1	1	0·2	*1·0*

This strain proved still transmissible fifteen months after the original infection.

Monkey 410: infected on 11. ii. 28 by flies: died 14. vi. 29.

Test	Date	Total flies dissected	Total infected flies	% of infected flies	No. of infected flies alive at 25th day: With glands infected	No. of infected flies alive at 25th day: Total	% of gland infections	Transmissibility index
1	2–22. iv. 28	291	3	1·0	0	3	0	*0*
2	27. v–16. vi. 29	299	0	0	0	0	0	*0*

STRAIN XXXIII (*T. GAMBIENSE*).

Monkey 544: infected by flies on 22. iv. 29: died 19. v. 30.

Test	Date	Total flies dissected	Total infected flies	% of infected flies	No. of infected flies alive at 25th day: With glands infected	No. of infected flies alive at 25th day: Total	% of gland infections	Transmissibility index
1	22. v–14. vi. 29	317	11	3·4	2	5	0·6	*1·3*
2	1–13. x. 29	310	3	0·9	2	3	0·6	*0·6*

Subinoculations into clean monkeys.

Monkey 713: inoculated from m. 544 on 16. v. 30.

Test	Date	Total flies dissected	Total infected flies	% of infected flies	No. of infected flies alive at 25th day: With glands infected	No. of infected flies alive at 25th day: Total	% of gland infections	Transmissibility index
1	29. v–21. vi. 30	447	19	4·2	12	15	2·6	*3·3*

Monkey 714: inoculated from m. 544 on 16. v. 30.

Test	Date	Total flies dissected	Total infected flies	% of infected flies	No. of infected flies alive at 25th day: With glands infected	No. of infected flies alive at 25th day: Total	% of gland infections	Transmissibility index
1	8–26. vi. 30	359	5	1·3	3	3	0·8	*1·3*

A year after its introduction into m. 544 the strain was still transmissible. At the time of the subinoculations m. 544 was very emaciated; it died three days later. In both these monkeys there has occurred a revival of the transmissibility of the strain as noted above with m. 95.

In the first test with m. 544 only five out of eleven flies survived twenty-five days, so that the true transmissibility index in this monkey was probably higher than is shown by the test.

Monkey 516: infected by syringe inoculation from the patient himself on 29. i. 29: died 23. ix. 30.

Test	Date	Total flies dissected	Total infected flies	% of infected flies	No. of infected flies alive at 25th day: With glands infected	No. of infected flies alive at 25th day: Total	% of gland infections	Transmissibility index
1	6–10. iii. 29	163	12	7·3	3	6	1·8	*3·6*

A poor test; only half the infected flies lived twenty-five days.

Subinoculations from m. 516 into clean monkeys.

Monkey 742: subinoculated on 2. viii. 30: died 14. xii. 30.

Test	Date	Total flies dissected	Total infected flies	% of infected flies	No. of infected flies alive at 25th day: With glands infected	No. of infected flies alive at 25th day: Total	% of gland infections	Transmissibility index
1	26. viii–19. ix. 30	680	23	3·3	12	13	1·7	*3·3*

The strain in m. 516 was still transmissible nineteen months after its original infection.

Monkey 727: subinoculated on 18. viii. 30: died 8. ix. 30.

Test	Date	Total flies dissected	Total infected flies	% of infected flies	No. of infected flies alive at 25th day: With glands infected	No. of infected flies alive at 25th day: Total	% of gland infections	Transmissibility index
1	29. viii–7. ix. 30	329	8	2·4	1	3	0·3	*0·8*

Only three out of eight positive flies lived twenty-five days; an unsatisfactory test. The monkey was in poor condition throughout.

Sheep 10: infected by flies from man on 26. ii. 29.

Test	Date	Total flies dissected	Total infected flies	% of infected flies	No. of infected flies alive at 25th day: With glands infected	No. of infected flies alive at 25th day: Total	% of gland infections	Transmissibility index
1	24. iii–17. iv. 29	237	3	1·2	1	1	0·4	*1·2*
2	15. xii. 30–12. i. 31	581	3	0·5	1	1	0·1	*0·5*

In each test the metacyclic index is unity.

Monkey 775: subinoculated from s. 10 on 25. x. 30, twenty months after the original infection of the sheep.

Test	Date	Total flies dissected	Total infected flies	% of infected flies	No. of infected flies alive at 25th day: With glands infected	No. of infected flies alive at 25th day: Total	% of gland infections	Transmissibility index
1	3–12. i. 31	582	6	1·0	3	6	0·5	*0·5*

Wild pig "Horace": infected by flies from m. 544 on 13. vii. 29.

Test	Date	Total flies dissected	Total infected flies	% of infected flies	No. of infected flies alive at 25th day: With glands infected	No. of infected flies alive at 25th day: Total	% of gland infections	Transmissibility index
1	25. vii–22. viii. 29	518	2	0·3	1	1	0·1	*0·3*
2	19. ix–6. x. 29	417	1	0·2	0	0	0	*0*
3	19. v–24. vi. 30	408	1	0·2	0	1	0	*0*
4	21. viii–17. ix. 30	406	0	0	0	0	0	*0*

On 3. vi. 30 (2) and 10. x. 30 (1) subinoculations of this pig's blood were made into clean monkeys, but the animals never became infected.

These experiments with the common wild bush pig of Uganda (*Potamochoerus choeropatmus*) should be compared with the experience of the Royal Society's Commission in 1910 when, after exposing a wild pig to flies infective with *T. gambiense*, one hundred clean *G. palpalis* were fed on the animal and 1 c.c. of its blood was inoculated into a white rat. No evidence of infection was obtained, and in the light of the present experiment this is not to be wondered at (Bruce, etc. 1911).

Later in the same year a young bush pig was injected on two occasions with the blood of monkeys infected with *T. gambiense*, and was also fed upon by three boxes containing flies known to be infective with this trypanosome. After a suitable interval two subinoculations from this pig were made into clean monkeys, and eighty-three clean flies were fed upon it, all with negative results (Fraser and Duke, 1910).

Taken all together these experiments show that although the bush pig can be infected and can infect *G. palpalis* with *T. gambiense*, this species of animal, which is exceedingly common throughout the Uganda Protectorate, is relatively resistant to the trypanosome.

STRAIN XXVII (*T. GAMBIENSE*).

Monkey 442: infected by flies on 30. iv. 28: died 7. xii. 29.

					No. of infected flies alive at 25th day			
Test	Date	Total flies dissected	Total infected flies	% of infected flies	With glands infected	Total	% of gland infections	Transmissibility index
1	14–25. vi. 28	365	8	2·1	3	7	0·8	*0·9*
2	11–28. viii. 28	331	6	1·8	3	5	0·9	*1·08*

Situtunga: (a half-grown male) infected by flies in mid-July, 1928, died 17. viii. 29.

					No. of infected flies alive at 25th day			
Test	Date	Total flies dissected	Total infected flies	% of infected flies	With glands infected	Total	% of gland infections	Transmissibility index
1	12. viii–2. ix. 28	402	1	0·2	0	1	0	*0*
2	10–28. x. 28	428	1	0·2	0	1	0	*0*
3	26. i–11. ii. 29	238	0	0	0	0	0	*0*

1068 flies were fed upon this antelope but no gland infections resulted.

STRAIN XXVIII.

Monkey 487: infected from man by inoculation by the syringe on 13. xi. 28.

					No. of infected flies alive at 25th day			
Test	Date	Total flies dissected	Total infected flies	% of infected flies	With glands infected	Total	% of gland infections	Transmissibility index
1	28. xi–18. xii. 28	293	2	0·6	0	1	0	*0*
2	29. i–14. ii. 29	240	12	5·0	5	5	2·0	*4·1*

This series is the most glaring exception that has yet been met to what may

be regarded as the normal procedure. The only explanation that offers itself is that the passage of the strain from man to this monkey by the syringe instead of by flies delayed the adjustment of the trypanosome in the monkey. It is to be noted that the first test was commenced the day after trypanosomes were first detected in m. 487's peripheral blood.

Strain XXIX "H" (*T. gambiense*).

This strain was from a European and proved to be non-transmissible by *G. palpalis* (Duke, 1930).

Monkey 507: infected by syringe, inoculated on 18. i. 29: died end of August, 1929.

Test	Date	Total flies dissected	Total infected flies	% of infected flies	No. of infected flies alive at 25th day: With glands infected	No. of infected flies alive at 25th day: Total	% of gland infections	Transmissibility index
*1	3–19. ii. 29	226	8	3·5	0	7	0	*0*
*2	5–24. iii. 29	385	9	2·3	0	7	0	*0*
*3	19. v–11. vi. 29	483	9	1·8	0	6	0	*0*

Monkey 507 was fed upon by infective flies carrying Strain XXVII on 11. vii. 29.

Test	Date	Total flies dissected	Total infected flies	% of infected flies	No. of infected flies alive at 25th day: With glands infected	No. of infected flies alive at 25th day: Total	% of gland infections	Transmissibility index
*4	22. vii–11. viii. 29	451	3	0·6	0	2	0	*0*

The attempt at superinfection apparently failed. But as with s. 218 above, it is possible that this test No. 4 was applied too soon after superinfection, and the protocol of this test lends some support to this suspicion.

Exp. no.	Infected animal	Day of experiment on which dissection began	No. of flies alive on 25th day	No. dissected during the experiment: Males	Females	Total	Duration of experiment in days	No. of flies containing flagellates: In gut only	In gut and salivary glands	Ages of the infected flies dissected during experiment	Dates of infecting feeds
421	m. 507	7	32	24	21	45	30	0	0	—	22–24. vii. 29
423	,,	6	43	26	24	50	28	0	0	—	23–25. vii. 29
426	,,	9	37	25	22	47	28	0	0	—	24–26. vii. 29
432	,,	7	32	18	28	46	28	0	0	—	27–29. vii. 29
435	,,	6	30	20	17	37	29	0	0	—	28–30. vii. 29
443	,,	9	27	28	14	42	28	1	0	,14,	31. vii–2. viii. 29
445	,,	6	31	25	25	50	29	1	0	,29,	1–3. viii. 29
451	,,	8	26	16	23	39	29	0	0	—	4–6. viii. 29
453	,,	7	42	35	18	53	30	1	0	,30,	5–7. viii. 29
462	,,	9	32	17	25	42	29	0	0	—	9–11. viii. 29

Monkey 607: infected by syringe inoculation on 30. ix. 29: died 13. ii. 30.

Test	Date	Total flies dissected	Total infected flies	% of infected flies	No. of infected flies alive at 25th day: With glands infected	No. of infected flies alive at 25th day: Total	% of gland infections	Transmissibility index
*1	15. x–11. xi. 29	375	6	1·6	0	5	0	*0*

Monkey 607 was superinfected by flies infective with Strain XXXIII on 8. xii. 29. The full protocol of the experiment is given, as it illustrates very clearly the delayed response that may follow superinfection, in this case in an animal infected originally with a non-transmissible strain.

(Superinfected by two positive gland flies from box 640 off 622 on 8. xii. 29.)

Exp. no.	Infected animal	Day of experiment on which dissection began	No. of flies alive on 25th day	No. dissected during the experiment: Males	Females	Total	Duration of experiment in days	No. of flies containing flagellates: In gut only	In gut and salivary glands	Ages of the infected flies dissected during experiment	Dates of infecting feeds
710	m. 607	5	16	15	22	37	28	0	0	—	19. xii. 29
711	,,	10	23	18	22	40	28	0	0	—	20–22. xii. 29
713	,,	10	34	16	27	43	27	0	0	—	21. xii. 29
714	,,	8	33	34	17	51	29	0	0	—	22–24. xii. 29
715	,,	9	38	23	21	44	28	0	0	—	23. xii. 29
717	,,	7	24	15	21	36	28	0	0	—	24. xii. 29
718	,,	6	29	24	11	35	30	0	0	—	25–27. xii. 29
720	,,	9	39	19	22	41	29	0	0	—	26–28. xii. 29
721	,,	8	30	18	16	34	28	0	0	—	27. xii. 29
723	,,	4	25	22	12	34	28	0	0	—	28. xii. 29
727	,,	6	28	22	16	38	29	0	0	—	29. xii. 29
729	,,	6	28	13	25	38	29	0	0	—	30–31. xii. 29
730	,,	7	40	24	27	51	28	0	0	—	31. xii. 29
734	,,	6	23	20	15	35	28	1	0	,28,	1–3. i. 30
735	,,	6	25	22	19	41	28	0	1	;28;	1–3. i. 30
737	,,	7	41	21	23	44	28	0	0	—	2–4. i. 30
740	,,	6	34	25	19	44	30	1	0	,7,	4–6. i. 30
764	,,	10	25	16	16	32	27	0	1	;25;	17–19. i. 30
765	,,	13	29	19	16	35	27	0	1	;27;	18–20. i. 30
766	,,	6	35	25	23	48	28	0	0	—	21–23. i. 30
767	,,	7	25	14	13	27	27	0	0	—	22–24. i. 30

Note. In these two protocols commas indicate that the enclosed figure relates to a gut infection, semi-colons to a gut and gland infection.

Test	Date	Total flies dissected	Total infected flies	% of infected flies	No. of infected flies alive at 25th day: With glands infected	Total	% of gland infections	Transmissibility index
1	19. xii. 29–24. i. 30	828	5	0·6	3	4	0·3	*0·4*

After this long and severe test blood from m. 607 was inoculated into clean m. 683 which died shortly afterwards, and from this animal clean m. 694 was inoculated on 6. iii. 30.

Note. The index of Strain XXXIII at the particular passage from which m. 607 was superinfected was *2·2*.

Monkey 694:

Test	Date	Total flies dissected	Total infected flies	% of infected flies	No. of infected flies alive at 25th day: With glands infected	Total	% of gland infections	Transmissibility index
1	26. iii–4. iv. 30	420	2	0·4	1	2	0·2	*0·2*

Monkey 506: infected by syringe inoculation on 8. iii. 29: died 30. ix. 29.

Test	Date	Total flies dissected	Total infected flies	% of infected flies	No. of infected flies alive at 25th day: With glands infected	No. of infected flies alive at 25th day: Total	% of gland infections	Trans-missibility index
*1	23. iii–18. iv. 29	343	4	1·1	0	3	0	*0*
*2	19. v–11. vi. 29	515	5	0·9	0	5	0	*0*

Strain XLVIII: Lakeshore strain.

Sheep 300: infected by fly on 13. ix. 27.

Test	Date	Total flies dissected	Total infected flies	% of infected flies	No. of infected flies alive at 25th day: With glands infected	No. of infected flies alive at 25th day: Total	% of gland infections	Trans-missibility index
1	23. x–8. xi. 27	210	11	5·2	5	8	2·4	*3·2*
2	28. v–13. vi. 28	504	6	1·1	3	6	0·5	*0·5*

Strain VI (*T. gambiense*).

Goat 341: infected by syringe on 12. v. 26: died 2. i. 28.

Test	Date	Total flies dissected	Total infected flies	% of infected flies	No. of infected flies alive at 25th day: With glands infected	No. of infected flies alive at 25th day: Total	% of gland infections	Trans-missibility index
*1	10. vii–6. viii. 26	235	6	2·5	0	5	0	*0*
*2	2. vi–16. vi. 27	200	1	0·5	0	1	0	*0*
*3	16. viii–1. ix. 27	304	1	0·3	0	1	0	*0*
	(Superinfected with Strain V on 22. viii. 27.)							
*4	5–28. ix. 27	455	0	0	0	0	0	*0*
	(Superinfected with Strain XLVIII on 15. x. 27.)							
*5	23. x–16. xi. 27	191	12	6·2	5	9	2·6	*3·4*

Strain II (*T. gambiense*).

Sheep 332: infected by syringe 31. iii. 26: died 12. i. 28.

Test	Date	Total flies dissected	Total infected flies	% of infected flies	No. of infected flies alive at 25th day: With glands infected	No. of infected flies alive at 25th day: Total	% of gland infections	Trans-missibility index
*1	1–30. v. 26	70	3	4·2	0	2	0	*0*
*2	5. vii–7. viii. 26	192	3	1·5	0	3	0	*0*
*3	17–30. iv. 27	223	4	1·7	0	4	0	*0*
	(Superinfected with Strain V on 25. viii. 27.)							
*4	7–28. ix. 27	422	1	0·2	0	1	0	*0*
	(Superinfected with *T. rhodesiense* on 13. xi. 27.)							
*5	24. xi–8. xii. 27	256	15	5·8	5	9	1·9	*3·2*

DISCUSSION.

The experiments given above show a remarkable agreement in testifying to a decrease in the transmissibility of *T. gambiense* when it remains for long in one and the same animal.

The infectivity of the strain to the gut of tsetse, apart from its transmissibility, also tends to diminish as the sojourn in the mammal increases.

The diminution in the adaptation of a strain to tsetse may progress to complete loss of transmissibility, and once this stage is reached it is highly improbable that transmissibility can ever be recovered. This opinion is supported by the behaviour of s. 161 and s. 333, by the study of a non-transmissible strain from man (Duke, 1930), and by work published in 1924 on the effect of prolonged syringe passage in trypanosomes.

On the other hand there is good reason to believe that in many cases the diminution is the effect of inhibitory influences exerted on the trypanosome in the course of time by the tissues of its mammalian host, and that the strain once freed from these influences by introduction into another and non-immune host may recover in this new host, temporarily at all events, its original transmissibility. This is suggested by the results of the subinoculations from monkeys 95, 544 and 516 into clean animals.

The transmissibility of a strain, though greatly reduced, may persist for a long time, often up to the death of the host. Thus trypanosomes in four sheep in these experiments were still infective to fly twelve, fifteen, fifteen and eighteen months respectively after their original infection. In two other sheep, which were tested not by fly feeds but by subinoculation into clean monkeys, transmissibility had persisted, apparently undiminished for fifteen months and two years and eight months respectively. In monkeys the results were similar. Two monkeys infected the glands of fly eighteen months and two two years and four months after their original infection respectively; and in two others, tested by subinoculation, the strain was still transmissible in one a year and in the other eighteen months later. We do not know whether these strains after their long stay in an individual mammal are still capable of infecting man, and it is hoped that some light may be thrown on this point by the investigations now being carried on at this laboratory by Dr A. R. D. Adams.

The additional evidence accumulated since the publication of the Commission's Report makes it therefore necessary to revise conclusions 2 and 5 of that document. It is true that most of this evidence has been obtained with two strains, Nos. V and XXXIII, which were selected for prolonged study mainly on account of their ready transmissibility by *G. palpalis*. But there is no reason to doubt that other strains if equally carefully studied would have behaved in the same way. It is at all events quite clear that *T. gambiense* can persist in a transmissible form in sheep for more than a year, and indeed until the death of the animal by some intercurrent disease or accident—for there is

no evidence that infection with strains of *T. gambiense* recently isolated from man necessarily shortens the life of sheep and goats. In the Report instances were described where individual animals showed complete resistance to infection with this trypanosome, and it is clear that some of the nineteen strains therein examined were much less able to survive in a transmissible form in domestic ruminants than were Strains V and XXXIII.

It is therefore highly probable that in addition to individual differences between different strains in their ability to utilise tsetse, individual mammalian hosts differ in their attitude towards the trypanosome, some being more congenial to it than others. Of the animals mentioned in this paper s. 333 and s. 332 appear to be examples of the uncongenial kind.

In nature revival of the transmissibility of a strain by the agency of inoculation into a new host must be an exceedingly rare event, even if it ever takes place. Direct transmission, *sensu strictu*, is essentially dependent on the presence of numbers of trypanosomes in the peripheral blood, and in the chronic infections we are considering this condition is in all probability never fulfilled.

There is at present little actual experimental evidence that cyclical passage through fly has the same stimulating effect on the transmission of the strain as direct inoculation into a new host. In the majority of the tests performed with longstanding infections the strain was not actually transmitted to and tested in a clean animal, the necessary information being supplied by dissection of the flies. In the case of s. 161, however, the strain was passed by tsetse to m. 422 after being sixteen months in the sheep.

The infectivity and transmissibility indices of the strain in the sheep at the time of this transmission were 1·5 and 0·5 and the corresponding figures for the monkey were 4·0 and 2·0.

This single experiment therefore suggests that the effect is much the same whatever method of passage is adopted, and we may assume that when a strain in a chronically infected host in nature is cyclically transmitted to a new host the transmissibility will be revived to a greater or less degree, the extent and permanency of the revival depending upon the amount of damage done to the strain in the previous host and also on the favourableness of the conditions which the trypanosome meets in its new environment.

From the standpoint of the epidemiologist these conditions suggest that the chief danger is the recently infected vertebrate host in which the trypanosome is best able to exercise to the full its powers of utilising tsetse.

Chronic cases, on the other hand, are very much less dangerous owing to the inhibition of the transmissibility of the trypanosome by prolonged contact with the tissues of one and the same host.

On the whole the experience of the last few years confirms conclusion 4 of the Commission's Report that the transmissibility of *T. gambiense* is greater in the human patient than in animals infected from him, whether infection be by fly-bite or by the syringe. This is additional experimental support of the

generally accepted view that *T. gambiense* is essentially a parasite adapted to man and dependent normally on man in nature.

Introduced from man into other hosts it is probable that a strain takes some time before it becomes fully adapted to its new environment, and during the period of inhibited transmissibility it is in grave danger of extinction. That similar changes take place in man himself we have reason to believe (Duke, 1930).

In three strains of polymorphic trypanosomes from natural sources other than man transmissibility by tsetse has been lost during a period of maintenance by syringe passage at this laboratory of many months' duration. Other strains isolated from man have been found to be non-transmissible in subinoculated animals, and in the case of one of them, Strain XXIX ("H"), the examination was exhaustive. Lastly on one occasion a strain, No. XXIII of the laboratory series, was obtained in a monkey which had been inoculated with blood from a situtunga on Damba Island, where buck and *G. palpalis* abound. This strain was found to be completely non-transmissible and all but non-infective to tsetse. The combined experience with all these strains suggests very strongly that the depression of transmissibility may and not uncommonly does proceed to the point where this property is permanently lost. The existence, so commonly reported by observers of Sleeping Sickness, of sporadic cases of human trypanosomiasis in fly-infested native populations, where the disease shows no signs of spreading, is thus easily explained. The trypanosome in such cases cannot pass cyclically through tsetse.

Superinfection.

For several reasons superinfection is important to the present discussion. First, it is an accident that must frequently befall both men and animals exposed to tsetse in nature; and secondly, by means of the transmissibility test, superinfection renders possible the recognition of a degree of immunity not appreciable by any test previously devised. And in demonstrating this immunity we demonstrate at the same time the utility of the method.

In the experimental section there are accounts of three sheep, a goat and four monkeys that were subjected to superinfection.

In the case of m. 95 successive infections led to successive revivals of the transmissibility of the strain. The greatest revival followed the introduction of a different strain of *T. gambiense* from that previously employed. With m. 375, which had been infected only four and a half months, superinfection produced no discernible change.

Monkeys 507 and 607 carrying a strain non-transmissible by tsetse gave interesting results. Monkey 507, superinfected with a transmissible strain after six months, failed to "take." It is just possible, however, that the test was invalid for reasons already given.

Monkey 607, originally infected on 30. ix. 29, was superinfected some two

months later and became, for the first time, infective to the glands of fly. Here the only suggestion of any immunity is the fall in the transmissibility index of the superinfecting strain from *2·2* before to *0·3* after its entry into m. 607, this level being maintained on subsequent inoculation into m. 694.

Turning now to the sheep. Sheep No. 161, superinfected with a homologous strain of *T. gambiense* fifteen months after first infection and at a moment when the original strain was still transmissible failed thereafter completely to infect the glands of fly. Trypanosomes were easily demonstrable on subinoculation of s. 161's blood into monkeys, but in these monkeys, also, the strain was non-transmissible.

Sheep 333, superinfected after sixteen months' contact with *T. gambiense*, failed to respond in any way. Four months later superinfection, this time with a heterologous strain of *T. gambiense*, again completely failed.

Sheep 332, infected originally with a feebly transmissible strain, never infected fly. Superinfection seventeen months later with a heterologous strain of *T. gambiense* failed to produce any change; but a second superinfection after a further two and a half months, this time with *T. rhodesiense*, was immediately established and rapidly killed the animal.

Goat 341 behaved in much the same way. After being infected for fifteen months with a non-transmissible strain of *T. gambiense*, superinfection with Strain V failed completely. Two months later superinfection with the Damba strain succeeded immediately, and the goat died of trypanosomiasis.

An association between two organisms that involves so many variable factors as does chronic trypanosomiasis is unlikely to adhere rigidly to a particular code of procedure. Nevertheless these studies of prolonged infection and superinfection with *T. gambiense* convey a strong suggestion that a definite biological principle governs the behaviour of these trypanosomes. It would appear that prolonged infection with *T. gambiense* leads to the development in the mammal of an immunity against this trypanosome.

This immunity acts first upon the transmissibility of the strain by tsetse and has no demonstrable effect on the mere presence of trypanosomes in the animal's circulation. The transmissibility is reduced to a low level and sometimes is abolished altogether.

There is reason to believe that whereas this immunity gradually develops in the course of an infection with a single strain, superinfection stimulates its production and intensifies its effect.

The application of this conception to naturally occurring trypanosomiasis is obvious. An infected animal is not necessarily infective to fly, and the constant exposure of adult animals to tsetse will tend sooner or later to their elimination from the circle of infection. The onus of responsibility for the spread of the trypanosome rests then with the young, in which the parasite enjoys its full transmissibility; and it is in fact from young game animals that trypanosomes are most readily obtained in actual experience. Once established, this immunity may persist for a long time, and it is possible that such an animal

will give a positive adhesion test for some time after it has ceased to harbour trypanosomes.

It is suggested that investigations upon the transmission by mosquitoes of the parasites of malaria, carried out on similar lines to those followed in the work outlined in this paper might yield information of both scientific and practical interest. There is already some evidence in the literature that strains of malaria may lose the power of forming gametocytes, and also that gametocytes are relatively less frequent in the blood of the adult than in the child population of hyperendemic and endemic areas. All these considerations suggest the possibility that similar laws may govern the behaviour of both these protozoa, and that immunity acquired by the vertebrate host may, sooner or later, reduce the transmissibility of the parasite by its normal insect vector.

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RED-CELL ADHESION IN TRYPANOSOMIASIS OF MAN AND OTHER ANIMALS.

II. SOME EXPERIMENTS ON THE MECHANISM OF THE REACTION.

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In a previous paper from this Institute (Duke and Wallace, 1930), red-cell adhesion in trypanosomiasis was described and an account given of some preliminary investigations on the mechanism of the test. The relationship between red-cell adhesion and the adhesion of platelets studied by Rieckenberg (1917), Davis and Brown (1927) and others, was considered and the belief expressed that "the two phenomena are different manifestations of the same substance in the plasma."

Red-cell adhesion can be demonstrated very simply by the mixing of equal parts of the citrated bloods of two monkeys, one having a chronic infection with some trypanosome which has almost completely vanished from the blood, or has been removed by treatment with drugs, and the other animal having an active infection with a trypanosome of the same group. Under the influence of a hypothetical substance, for which the term "adhesin" was suggested by Duke and Wallace, red blood corpuscles adhere to the trypanosomes and in a strongly positive reaction all the trypanosomes are completely enveloped with red cells.

The study of the mechanism of red-cell adhesion made in the previous work (*loc. cit.*) was concerned with attempts to separate the various components of the reaction and to determine which components were essential for the phenomenon. Many difficulties were encountered, these being most evident when it was desired to obtain trypanosomes free from plasma. The preliminary experiments indicated, however, that to obtain red-cell adhesion it was necessary to have at least three components: (1) red blood cells of a primate; (2) the plasma or serum of an animal which had had trypanosomiasis and preferably one with a long-standing infection or an infected animal which had been treated with trypanocidal drugs (this plasma or serum supplies the adhesin), and (3) the trypanosome-containing plasma, this trypanosome being related to that which produced the adhesin.

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No absolute specificity in this adhesion phenomenon could be observed and in addition it was found that a single or even a number of negative observations do not exclude trypanosomiasis. A positive reaction, however, invariably indicated in these experiments infection with trypanosomes of the same group but not necessarily trypanosomes specifically identical with those employed in the actual test. Thus *gambiense* adhesin bloods not uncommonly reacted with *rhodesiense* trypanosomes.

For field purposes, the red-cell adhesion test is most suitable and in view of a possible further application, either in the original or in a modified form, it was thought desirable to make a more intensive study of the mechanism of the test. For ordinary practical applications, the original test involving the simple mixture of two citrated bloods is all that is necessary, but for the work described here several modifications have been introduced. Washed human red blood cells of an individual of blood group O have been used wherever possible, thus avoiding the haemagglutination which would occur on admixture with certain human and monkey sera. In addition, a more or less constant supply of adhesin was required for this work, and therefore selected monkeys were bled at a time when the serum showed a good adhesin content; these sera, after filtration through a Berkefeld filter candle, showed a good adhesin titre for at least several months, and each serum could thus be used for a large number of tests. In this way the progress of the work was not impeded by any variation which might have occurred in the adhesin content of the serum or plasma of the adhesin animal. Finally, the work of Schern (1925) and the more recent work of Yorke, Adams and Murgatroyd (1929 and 1930) has shown that trypanosomes can be kept alive for a much longer period of time if glucose is added to the medium, and by the use of a citrate-Ringer-glucose solution similar to that recommended by the latter workers it has been found possible to wash trypanosomes free from plasma and use them for adhesion purposes.

TECHNIQUE.

Red-cell suspension.

Six or seven drops of blood were collected in about 10 c.c. of citrate-Ringer-glucose solution[1] and the mixture shaken and centrifuged. The red-cell deposit was thoroughly mixed with about 10 c.c. of citrate-Ringer-glucose solution, centrifuged again and the deposit suspended in about 1 c.c. of citrate-Ringer-glucose solution. Unless otherwise stated the cells were those of apparently normal animals.

Adhesin solutions.

(*a*) *Adhesin serum.* Monkeys infected with *T. gambiense* or *T. rhodesiense* were treated with Bayer 205, and when the plasma or serum showed a good adhesin content the animals were bled and the serum obtained. Other sera used were those of human sleeping sickness patients and in some instances sera

[1] This solution was the Ringer-glucose solution recommended by Yorke, Adams and Murgatroyd (1930, p. 124) with the addition of sodium citrate to give 1 per cent.

of untreated monkeys with a chronic trypanosome infection. Most of the adhesin sera were filtered through Berkefeld filter candles and were kept in sealed tubes in an ice-box. They maintained a good adhesin content for at all events several months.

(*b*) *Adhesin plasma* (*diluted*). In many experiments it was found desirable to use fresh adhesin plasma. The whole blood of the animal (usually an infected and "bayerised" monkey) was mixed with twice its volume of citrate-Ringer-glucose solution, the mixture centrifuged and the clear supernatant solution used.

Trypanosome suspensions.

(*a*) *Diluted trypanosome plasma.* One volume of the whole blood of the infected animal (monkey or guinea-pig) was thoroughly mixed with two volumes of citrate-Ringer-glucose solution, the mixture centrifuged slowly to remove the red cells, and the supernatant trypanosome-containing solution used for the tests.

(*b*) *Washed trypanosomes.* The diluted tryp. plasma (1 c.c.), obtained as described above, was centrifuged at high speed for about 15 minutes, the supernatant solution pipetted off and the deposit of trypanosomes (and some red cells) mixed well with citrate-Ringer-glucose solution (approximately 1 c.c.). This suspension was centrifuged slowly to remove the majority of the red cells present and the remaining suspension centrifuged at high speed for 15 minutes. The supernatant solution was again discarded and the trypanosome deposit suspended in citrate-Ringer-glucose solution, the final volume being somewhat less than that of the diluted tryp. plasma used for the preparation of these washed trypanosomes.

Incidentally it is of interest to note that further washing with citrate-Ringer-glucose solution and the necessary centrifuging do not materially damage the trypanosomes for red-cell adhesion, and complete or almost complete adhesion can be obtained with these "super-washed" trypanosomes provided that the other factors essential for the reaction are present.

Diluted normal plasma.

A mixture of one volume of whole blood and two volumes of citrate-Ringer-glucose solution was centrifuged at high speed and the supernatant diluted plasma pipetted off.

The red-cell adhesion test.

One drop each of the necessary solutions (adhesin plasma or serum, tryp. plasma and red-cell suspension in the earlier tests) were mixed in a small test tube (2 in. × ¼ in.) and allowed to stand at room temperature (24–27° C.). Citrate-Ringer-glucose solution was added where necessary to make the volume the same in each tube. After 15 minutes, drops of the mixture were transferred to microscope slides, covered with cover-slips and observed under

the microscope. During the next 10 minutes the free trypanosomes and the trypanosomes with red cells adhering to them were counted by two observers independently and the result expressed as a percentage of adhering trypanosomes to total trypanosomes counted. In practically all the experiments quoted in this paper, when adhesion occurred it was of the "caddis-form" type previously described (Duke and Wallace, 1930).

These details of the test were fixed after preliminary investigation had been made to determine as far as possible the optimum conditions for the test and the significance to be attached to the results of the counting. The total number of trypanosomes counted by each observer was usually between 60 and 150 (average 92) and the agreement between the figures obtained for percentage adhesion was most satisfactory. Where adhesion occurred, the average deviation from the mean value was slightly less than 2 per cent. and the maximum deviation 7·5 per cent.

Details of the adhesin solutions and trypanosome suspensions referred to in the tables appended.

The experiments described below are typical experiments selected from a large number carried out with various adhesin solutions and trypanosome suspensions. The serum and plasma of man and monkeys were the sources of adhesin, and the trypanosome suspensions were obtained from infected monkeys (*T. rhodesiense* and *T. gambiense*) and guinea-pigs (*T. rhodesiense* and *T. gambiense*). The results of the test with *T. rhodesiense* and a *rhodesiense* adhesin were apparently identical in all respects with those with *gambiense* trypanosomes and a *gambiense* adhesin.

In the experiments quoted in the tables the following solutions were used:

Adhesin serum 761. Monkey 761 was infected with *T. rhodesiense*, treated with Bayer 205 and bled at a time when the plasma showed a good adhesin titre. The serum was filtered through a Berkefeld candle and kept in an ice chest.

Adhesin plasma 744. Monkey 744 was infected with *T. rhodesiense* and was later "bayerised." The animal was kept alive and its diluted plasma used for several experiments. The adhesin content of the plasma varied considerably from time to time (cf. Duke and Wallace, 1930).

Human adhesin serum (*Osanda*). This was the serum of a native trypanosomiasis patient (*T. gambiense*), the infection being apparently resistant to treatment with tryparsamide.

Tryp. plasmas 768, 782, 786 *and* 787 (*monkeys*). The diluted trypanosome-containing plasmas of monkeys infected with *T. rhodesiense.*

Tryp. plasma G (*guinea-pig*). A *gambiense* infection.

Recording of the results.

In this work it has not been found necessary to differentiate between various degrees of adhesion, and the results under adhesion are given as

"strong" or "none." In addition, actual figures are given for the percentage adhesion obtained in typical experiments.

Experimental.

The adhesion reaction with red cells of various animals.

The observations of Duke and Wallace (1930) showed that the phenomenon of red-cell adhesion is in some way associated with the presence of the red corpuscles of primates. The red cells of a large number of different animals were tested, but only those of man, monkey and baboon would adhere to trypanosomes. It was thought desirable to carry out similar experiments with the modified technique used in this work and using various adhesin sera (human and monkey). In addition, it was found possible to test the red cells of a few animals which were not investigated on the previous occasion.

The results of these experiments, some of which are recorded in Table I, offered a complete confirmation of the statement that for adhesion of this type the red cells of some primate are required. It must be noted, however, that with the animals used for these tests no positive reaction was obtained with the red corpuscles of infected guinea-pigs, as was observed in the earlier work. In the course of other investigations, however, mainly in connection with sheep adhesins, adhesion of the red cells of infected guinea-pigs has been occasionally obtained, and in these instances no adhesion occurred with the red cells of non-infected guinea-pigs or sheep. This aspect of red-cell adhesion is being investigated further.

Table I. *Adhesion with the red cells of various species.*

Cells	Adhesin solution	Trypanosomes	Adhesion
Human	Monkey serum (761)	Diluted monkey plasma (787)	Strong (87 %)
Monkey	,, ,, ,,	,, ,, ,, ,,	Strong (85 %)
Baboon	,, ,, ,,	,, ,, ,, ,,	Strong (88 %)
Calf	,, ,, ,,	,, ,, ,, ,,	None (0 %)
Goat	,, ,, ,,	,, ,, ,, ,,	None (0 %)
Hen	,, ,, ,,	,, ,, ,, ,,	None (0 %)
Human	Monkey serum (761)*	Diluted monkey plasma (768)	Strong (89 %)
Monkey	,, ,, ,,	,, ,, ,, ,,	Strong (92 %)
Guinea-pig	,, ,, ,,	,, ,, ,, ,,	None (2 %)
Infected guinea-pig	,, ,, ,,	,, ,, ,, ,,	None (0 %)
Human	Human serum (Osanda)	Diluted guinea-pig plasma (G)	Strong (64 %)
Guinea-pig	,, ,, ,,	,, ,, ,, ,,	None (0 %)
Lizard	,, ,, ,,	,, ,, ,, ,,	None (0 %)
Mouse	,, ,, ,,	,, ,, ,, ,,	None (0 %)

* For this experiment, serum 761 was previously treated with guinea-pig red cells to remove from it agglutinins for these cells.

In several of these experiments with human and monkey adhesin sera marked agglutination of the red cells occurred, and this prevented any satisfactory reading being made of the extent of adhesion, if any. Thus it was felt desirable that the agglutinins concerned should be removed, and preliminary experiments were carried out to determine whether this was possible without

a simultaneous removal of the adhesin. In one of these experiments (cf. Table I) monkey adhesin serum 761, which had a marked agglutinating action on guinea-pig red cells, was treated with washed guinea-pig red cells and left, with occasional shaking, at room temperature (25° C.) for about 45 minutes. The cells were then removed by centrifuging and the clear supernatant solution tested for adhesin, when it was found that the agglutinins had been almost completely removed, whereas the adhesin content was practically unchanged. Similar experiments, described more fully later, showed that the blood group agglutinins (α and β) can be absorbed from a human adhesin serum of group O without producing any marked lowering of the adhesin content; in the same way, treatment of a monkey adhesin serum with the mixed washed red cells of human, monkey, baboon and rabbit bloods removes the corresponding agglutinins for these cells but not the adhesin (cf. Table VI). This removal of red-cell agglutinins without loss of adhesin enabled tests to be made with various red cells which could not be used in the simple adhesion test because of the marked agglutination. In no instance, however, was adhesion observed with cells other than those of primates.

Whenever possible the experiments described below were carried out with human red cells and usually those of a member of blood group O (for nomenclature, see review by Landsteiner, 1928) in order to avoid agglutination by human adhesin sera.

Red-cell adhesion with washed trypanosomes.

For a complete analysis of the red-cell adhesion test it is desirable to have the various components—red cells, adhesin and trypanosomes—free, as far as possible, from other constituents of the blood. In the experiments so far described, this condition could only apply to the red cells, which were removed from the plasma and were thoroughly washed with citrate-Ringer-glucose solution. Attempts were now made to use for the test trypanosomes which had been washed in a similar way, but although success was occasionally achieved, in most cases no adhesion was obtained. The trypanosome-containing diluted plasma was centrifuged at high speed to bring down the trypanosomes, the deposit suspended in citrate-Ringer-glucose solution and centrifuged again. The washed trypanosome deposit was suspended in citrate-Ringer-glucose solution and in this suspension the trypanosomes were very active and would remain alive for at least several hours but would give no adhesion with a filtered adhesin serum. It may be noted here that Regendanz and Jurukoff (1930) failed to obtain either platelet adhesion to trypanosomes or trypanosome agglomeration when centrifuged trypanosomes were used and these authors attribute this fact to the injurious action of the centrifuging.

In spite of these failures, it was felt, however, that this inability of washed trypanosomes (and of trypanosomes which had simply been removed from the plasma by centrifuging) to give the red-cell adhesion phenomenon could not be attributed to mere alteration in properties due to mechanical treatment, and

a simple experiment confirmed this view. A trypanosome-containing plasma was divided into two portions and both were centrifuged at high speed for 15 minutes. The supernatant solution in one tube was drawn off and replaced with citrate-Ringer-glucose solution, and the tube shaken (preparation "A"); the second tube, the control, was merely shaken to remix the contents (preparation "B"). When tested with human red cells and a filtered monkey adhesin serum, trypanosome preparation "A" gave no adhesion whereas preparation "B" was just as effective as the original tryp. plasma. Experiments involving more drastic treatment confirmed this observation that centrifuging, *per se*, does not to any appreciable extent injure trypanosomes for adhesion purposes.

The conclusion reached as a result of these experiments with washed trypanosomes was that in addition to the adhesin there is some other factor necessary for red-cell adhesion and that this factor is present in the trypanosome-containing plasma. Several experiments were then carried out to establish this point and Table II gives some typical results.

Table II. *Adhesion with washed trypanosomes.*

Cells	Adhesin	Trypanosomes	"X" factor	Adhesion
Human	None	Diluted monkey plasma (782)	None	None (0%)
,,	Monkey serum (761)	,, ,, ,, ,,	None	Strong (61%)
,,	,, ,, ,,	Washed tryps. of 782	None	None (0%)
,,	,, ,, ,,	,, ,, ,, ,,	Diluted human plasma	Strong (80%)
,,	,, ,, ,,	,, ,, ,, ,,	Diluted guinea-pig plasma	Strong (65%)
,,	None	,, ,, ,, ,,	,, ,, ,,	None (0%)
Human	Diluted plasma 744	Diluted plasma 782	None	Strong (91%)
,,	,, ,, ,,	Washed tryps. of 782	None	Fairly strong (46%)
,,	,, ,, ,,	,, ,, ,, ,,	Diluted human plasma	Strong (74%)
,,	,, ,, ,,	,, ,, ,, ,,	Diluted guinea-pig plasma	Strong (85%)

From the first half of this table it will be seen that washed trypanosomes gave no adhesion with a filtered adhesin serum but would readily do so if a little normal human or guinea-pig (or monkey) plasma was added. No adhesion was obtained in the absence of the adhesin serum. Thus four components appear to be necessary for the red-cell adhesion phenomenon: red cells (of a primate), the adhesin, trypanosomes and some constituent ("X" factor) of normal serum (human, monkey or guinea-pig, etc.). The last-named constituent would, of course, be present in the adhesin serum originally, but had been removed during filtration through the Berkefeld filter. If these conclusions were justified, washed trypanosomes should give adhesion with a fresh adhesin plasma or serum without further addition of the "X" factor, and the experiment recorded in the second half of Table II showed this to be true. The same washed trypanosomes which gave no adhesion with a filtered adhesin serum gave good adhesion with a fresh adhesin plasma. The adhesion was not maximal however, presumably because of the considerable dilution of the adhesin plasma (ultimate concentration of 1 in 12) and the percentage adhesion could be raised considerably by the addition of a little fresh normal serum.

Effect of heat on the "X" factor.

Since this "X" factor necessary for red-cell adhesion appears to be present in most normal sera and since it can readily be removed by filtration of the serum through a Berkefeld filter, it was immediately thought that this agent might be complement, and experiments were carried out to determine whether it was resistant to the action of heat. From the results given in Table III the conclusion can be reached that the "X" factor resembles haemolytic complement in being destroyed by 30 minutes heating at 56° C. The adhesin serum used for the experiment quoted was a filtered serum and had no haemolytic complement activity, whilst haemolytic tests were carried out to prove that the heating process had really destroyed the complement present in the guinea-pig and human plasma. In addition it was shown that a mixture of the adhesin serum and heated human (or guinea-pig) plasma had no haemolytic complement action.

Similar experiments were carried out with a fresh adhesin serum (Table IV), a serum which was quite rich in haemolytic complement. Heating at 56° C. for 30 minutes destroyed the complement power but did not affect the adhesin, and this heated adhesin serum behaved in all respects like a "filtered" adhesin serum. Thus with washed trypanosomes the unheated adhesin serum gave good adhesion, whereas the heated serum gave none unless a little fresh normal plasma or serum was added. Heated human or guinea-pig plasma or serum could not reactivate the heated adhesin serum.

The action of ammonia on the "X" factor and on the adhesin.

Gordon, Whitehead and Wormall (1926) have shown that dilute ammonia has a destructive action on haemolytic complement, resulting in the almost specific inactivation of a relatively heat-stable fourth component. It was thought of interest, therefore, to determine how far the "X" factor necessary for red-cell adhesion resembles complement in its behaviour with respect to treatment with ammonia. The process of inactivation was essentially that described previously (Gordon, Whitehead and Wormall, 1926): one volume of approximately $N/6$ NH_4OH was added to four volumes of guinea-pig (or monkey) serum or diluted plasma and the mixture was kept at 37° C. for about $1\frac{1}{2}$ hours, after which $N/8$ HCl was added to bring the reaction to approximately *p*H 7·5. The untreated plasma or serum used for comparison was diluted to a corresponding extent with 0·85 per cent. sodium chloride solution.

The results of these experiments (cf. Table V) indicate a very close similarity between haemolytic complement and the "X" factor necessary for red-cell adhesion, for both are readily destroyed by treatment with dilute ammonia solution.

Gordon and Wormall (1928) have shown that the bactericidal action of normal guinea-pig serum on *B. dysenteriae* (Flexner) appears to be due to the presence of an immune body, which is relatively resistant towards ammonia,

Table III. *Effect of heat on the "X" factor or complement-like substance.*

Cells	Adhesin	Trypanosomes	"X" factor	Adhesion
Human	Monkey serum (761)	Washed tryps. of 782	None	None (0 %)
,,	,, ,, ,,	,, ,, ,, ,,	Diluted guinea-pig plasma	Strong (63 %)
,,	,, ,, ,,	,, ,, ,, ,,	,, ,, ,, (heated at 56° C.)	None (0 %)
,,	,, ,, ,,	,, ,, ,, ,,	Diluted human plasma	Strong (78 %)
,,	,, ,, ,,	,, ,, ,, ,,	,, ,, ,, (heated at 56° C.)	None (0 %)
,,	None	,, ,, ,, ,,	Diluted human plasma	None (0 %)

Table IV. *Effect of heat on the "X" factor and on the adhesin.*

Cells	Adhesin	Trypanosomes	"X" factor	Adhesion
Human	Human serum (Osanda)	Diluted plasma (guinea-pig)	None	Strong (88 %)
,,	,, ,, ,,	Washed tryps. of guinea-pig	None	Strong (71 %)
,,	Heated* ,, ,, ,,	,, ,, ,, ,,	None	None (0 %)
,,	Heated* ,, ,, ,,	,, ,, ,, ,,	Diluted human plasma	Strong (68 %)
,,	Heated* ,, ,, ,,	,, ,, ,, ,,	Heated* ,, ,, ,,	None (2 %)
,,	Heated* ,, ,, ,,	,, ,, ,, ,,	Diluted guinea-pig plasma	Strong (82 %)
,,	Heated* ,, ,, ,,	,, ,, ,, ,,	Heated* ,, ,, ,,	None (1 %)

* Heated at 56° C. for 30 minutes.

Table V. *The action of ammonia on the "X" factor or complement-like substance.*

Cells	Adhesin	Trypanosomes	"X" factor	Adhesion
Human	Monkey serum (761)	Washed tryps. of monkey 786	None	None (0 %)
,,	,, ,, ,,	,, ,, ,, ,, ,,	Monkey plasma	Strong (97 %)
,,	,, ,, ,,	,, ,, ,, ,, ,,	,, ,, (NH_3-treated)	None (0 %)
Human	NH_3-treated serum 761	Washed tryps. of monkey 786	None	None (0 %)
,,	,, ,, ,,	,, ,, ,, ,, ,,	Monkey plasma	Strong (98 %)
,,	,, ,, ,,	,, ,, ,, ,, ,,	,, ,, (NH_3-treated)	None (0 %)

Haemolytic tests: serum 761, NH_3-treated monkey plasma and a mixture of the two had no significant haemolytic complement action.

and a bactericidal complement, which is readily destroyed by ammonia; and since red-cell adhesion requires the presence of an adhesin, which has certain similarities to a true antibody, and a complement-like substance, it was expected that the latter only would be destroyed by ammonia. The results of experiments along these lines (cf. the second part of Table V) furnished evidence in favour of this view. The adhesin does not appear to undergo any significant destruction as a result of treatment with sufficient ammonia to destroy the haemolytic complement and the complement-like "X" factor necessary for red-cell adhesion.

These experiments have been repeated with guinea-pig plasma as the source of the "X" factor with results essentially identical with those given in Table V.

Relationship between red-cell adhesion and haemagglutination.

Experiments were carried out to determine whether there exists any relationship between the adhesion of human red cells to trypanosomes under the influence of an adhesin serum and agglutination of those cells by a human serum containing the necessary blood group agglutinins. A human adhesin

Table VI. *Effect of absorption from the adhesin serum of agglutinins for various red cells.*

Cells	Adhesin	Trypanosomes	"X" factor	Adhesion
Human	Absorbed* monkey serum (761)	Washed tryps. (787)	Diluted human plasma	Strong (83 %)
Monkey	" " " "	" " "	" monkey "	Strong (89 %)
Baboon	" " " "	" " "	" baboon "	Strong (94 %)
Rabbit	" " " "	" " "	" rabbit "	None (0 %)
Human	None	" " "	" human "	None (0 %)
"	Unabsorbed 761 serum	" " "	" " "	Strong (91 %)
"	" " "	" " "	None	None (0 %)

* This serum was obtained as follows: serum 761 was added to a centrifuged deposit of mixed washed human (Group O), monkey, baboon and rabbit cells, the mixture thoroughly shaken and left, with occasional shaking, at room temperature (25·5° C.) for 1 hour, after which it was centrifuged and the clear supernatant solution used.

serum of an individual of blood group O and thus containing both α and β agglutinins was absorbed separately with washed human red cells of (i) group O (ii) group A and (iii) group B. In (ii) almost complete removal of the α agglutinin and in (iii) removal of the β agglutinin occurred, but in neither case, nor as a result of absorption with group O red cells, was there any significant loss of adhesin. Similarly, by absorption with the mixed red cells of group A and group B individuals the removal of both α and β isohaemagglutinins was effected without appreciable loss of adhesin.

Human and monkey sera often contain agglutinins for the red cells of other species, and attempts were made to find out whether these agglutinins play any part in red-cell adhesion to trypanosomes. From the experiment recorded in Table VI it will be seen that absorption of a monkey adhesin serum with mixed washed human, monkey, baboon and rabbit red cells did not appreciably

reduce the adhesin content, whereas the agglutinins for monkey, baboon and rabbit cells were for all practical purposes completely removed. For this experiment washed trypanosomes were used and the "X" factor or complement-like substance was supplied by the addition of the corresponding diluted plasma, *i.e.* human plasma for the tests with human red cells.

From these experiments the conclusion is reached that neither the isohaemagglutinins, nor the agglutinins for the red cells of other species, play any significant part in red-cell adhesion. It is of interest to note that Regendanz and Jurukoff (1930) have recently found no relationship between platelet adhesion to trypanosomes and blood group agglutination.

Discussion.

No attempt has been made in this work to examine the relationship between the red-cell adhesion phenomenon and the platelet adhesion first described by Rieckenberg (1917). This relationship was discussed fully by Duke and Wallace (1930) who concluded that the evidence is in favour of a very close similarity, and that there seems to be justification for "the belief that the two manifestations are merely different expressions of the same physiological state." There are, however, one or two facts which do not appear to support this view, for whereas in the platelet test the platelets merely serve as an indicator and can be replaced by bacteria (Brussin, 1925; Krantz, 1926; Davis and Brown, 1927) and by colloidal inorganic suspensions and such indicators as gamboge (Davis and Brown, 1927), red-cell adhesion is more specific and occurs with the red cells of primates only. It is hoped that a further study of this question will be possible in the near future.

Perhaps the most interesting conclusion to be drawn from the experiments described here is concerned with the action of complement or some closely related factor. Several authors have studied the question of the importance of complement in the other adhesion reactions—adhesion of platelets, leucocytes and bacteria to trypanosomes and leptospira. Thus Kritschewski and Tscherikower (1925) stated that free complement is necessary for platelet adhesion to trypanosomes, and although Davis and Brown (1927) were unable to satisfy themselves that thermolabile complement is essential for this reaction, they found it necessary for adhesion of bacteria to leptospira (Brown and Davis, 1927). Regendanz and Jurukoff (1930) have more recently investigated the importance of complement in platelet adhesion and trypanosome agglomeration, phenomena which, according to these authors, are due to the same antibodies. They were unable to make a definite decision on this point, their evidence tending to show that complement is not necessary.

In this paper experiments are described which show that filtration of an adhesin serum through a Berkefeld filter candle, a process which, in the earlier stages at least, removes complement (Muir and Browning, 1909), leads to the failure of this adhesin serum to give red-cell adhesion with washed trypano-

somes and human (or monkey) red cells. No appreciable loss of adhesin occurs as a result of this filtration and in this respect the adhesin behaves like other immune bodies. The reactivation by the addition of a little normal human, monkey, baboon, guinea-pig or rabbit serum or plasma indicates that some complement-like substance present in most, if not all, normal sera is necessary for red-cell adhesion. This activating power of normal serum is lost when the serum is heated to 56° C. for 30 minutes, and it is also destroyed when the serum is acted upon by dilute ammonia, as is haemolytic complement (Gordon, Whitehead and Wormall, 1926). Thus in these respects, *i.e.* removal by filtration through a Berkefeld filter, destruction when the serum is heated at 56° C. and inactivation by ammonia, haemolytic complement and the "X" factor behave identically. From experiments not quoted in full in this paper, it has been found, however, that there is no strict parallelism between the haemolytic complement activity of a serum and the power to "activate" washed trypanosomes for adhesion purposes; thus in tests involving dilution series with human, monkey and guinea-pig plasmas, it was found that the most active serum for haemolytic purposes was not always that which induced the best red-cell adhesion. There are probably several other factors, such as antihaemolytic effects, which might have to be considered here and these might account for the discrepancy. It is perhaps too much to expect that there should be an absolute parallelism between the two activities. Thus all the available evidence points to the necessity of some complement-like substance for red-cell adhesion, but more evidence will be required before it can be stated with certainty that the "X" factor is the same as complement. On the other hand, no evidence against the identity has been obtained, with the exception of the discrepancy above noted, and it is considered unnecessary to assume the presence in normal serum of a special "adhesin" complement.

A note on "auto-adhesion."

On certain, rather rare, occasions some adhesion has been observed in the control tests where the diluted monkey "tryp. plasma" was added to normal human or monkey red cells, indicating that the infected blood contained adhesin as well as trypanosomes. Adhesion was likewise obtained when these infected bloods were simply diluted with citrate-Ringer-glucose solution, and there appears to be no doubt that adhesin and trypanosomes can be present in the blood at the same time. This phenomenon of "auto" red-cell adhesion has been observed with the diluted blood of a *gambiense* monkey which had relapsed after treatment with atoxyl, and also in the case of a few untreated infected monkeys, but it occurred only in a small number of the bloods examined, usually when relatively few trypanosomes were present in the blood. Adhesion was of the "caddis" type, and it is considered possible that some of the instances of non-specific adhesion reported by Duke and Wallace (1930, p. 419) can be explained in this way. In the majority of cases where non-

specific adhesion was noted, however, tests involving the addition of the same "tryp. plasma" to other bloods gave perfectly negative results, and here the possibility of "auto-adhesion" can be discounted.

Summary.

1. The red-cell adhesion phenomenon in trypanosomiasis of man and other animals, first described by Duke and Wallace (1930) and shown by these authors to be due to the presence in the blood of the infected animal of a substance (adhesin) which appears during the course of an infection, has been investigated further. Several strains of *T. rhodesiense* and *T. gambiense* have been used for this study.

2. Red-cell adhesion of this nature has been obtained with the red blood cells of primates only, thus confirming the earlier finding.

3. No relationship appears to exist between red-cell adhesion and isohaemagglutination, or other types of haemagglutination. The removal, by absorption, of the α and β isohaemagglutinins from a human adhesin serum of blood group O, or the removal from a monkey adhesin serum of agglutinins for baboon, rabbit and guinea-pig red cells does not lead to the removal of the adhesin.

4. Centrifuging, *per se*, does not destroy to any appreciable extent the power of trypanosomes to adhere to red cells.

5. Trypanosomes which have been freed from plasma by centrifuging and subsequent washing with citrate-Ringer-glucose solution give good adhesion with human or monkey red cells when a fresh adhesin serum or plasma is used. Little or no adhesion is obtained with these washed trypanosomes however, if the adhesin serum is very old or if it has been previously filtered through a Berkefeld filter candle or if the adhesin serum or plasma has been heated at 56° C. for 30 minutes.

6. From this and other evidence the conclusion is reached that in addition to the red cells, trypanosomes and the adhesin some other factor (designated the "X" factor) is necessary for red-cell adhesion.

7. This "X" factor is present in the serum or plasma of humans, monkeys, baboons, rabbits and guinea-pigs and has properties similar to those of complement. It is removed, as is most of the haemolytic complement, when the serum is filtered through a Berkefeld filter, it is destroyed by heating at 56° C. for 30 minutes, and, like haemolytic complement, it is inactivated when the serum is subjected to the action of dilute ammonia for about $1\frac{1}{2}$ hours. Filtration, heating at 56° C. for 30 minutes and dilute ammonia appear to have no significant destructive action on the adhesin.

8. The requirements for this red-cell adhesion phenomenon are (*a*) the red cells of a primate, (*b*) an adhesin, which is probably an antibody-like substance produced during infection with trypanosomes, (*c*) trypanosomes of a strain related to that which gave rise to the formation of the adhesin, and

(*d*) a complement-like component ("X" factor) present in the plasma and serum of most, if not all, normal animals.

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THE MORPHOLOGY AND LIFE HISTORY OF THE FOWL NEMATODE *ASCARIDIA LINEATA* (SCHNEIDER)[1].

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(With Plates XIII, XIV, and 25 Text-figures.)

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Taxonomy.

The finding of Schwartz, in 1925, that *Ascaridia lineata* (Schneider) is the usual large nematode of the chicken in the United States and the recognition by Cram (1927) of *Ascaridia galli* (Schrank 1788) Freeborn 1923 as a species of fowl helminth led to my studying a larger number of *Ascaridia* than was planned for the morphological studies in progress and to my consulting the relevant literature. While examining the early descriptions of *Ascaris galli* Schrank 1788, *Ascaris perspicillum* Rudolphi 1803, *Ascaris inflexa* (Zeder 1800) Rudolphi 1809 and *Heterakis lineata* Schneider 1866, it became evident that Schneider's characterisation including lateral lines and a drawing of *H. lineata* affords the first adequate description of the parasite under consideration, the number and arrangement of the caudal papillae (male) having been portrayed then as they are now recognised in *A. lineata* (Text-fig. 10, p. 365). Dujardin's (1845) creation of the genus *Ascaridia* and its adoption for this species by Railliet and Henry (1912) make its name *Ascaridia lineata* (Schneider 1866) Railliet and Henry 1912. The characters of the *Ascaridia* I have studied are in accord with those shown by Schneider (1866) and so

[1] Contribution No. 134 from the Department of Zoology, Agricultural Experiment Station, Kansas State College of Agriculture and Applied Sciences, Manhattan, and from the Molteno Institute for Research in Parasitology, University of Cambridge, England.

nearly in agreement with those of Schwartz for this parasite that I conclude that the large fowl nematodes which occur in Kansas and about Cambridge (England) are *A. lineata*.

Described from material collected in Brazil, it probably was again reported from that country by Magalhães (1892) as *A. braziliensis* which Travassos (1913) considers to be a synonym of *A. lineata*. Von Linstow (1883) reported *H. lineata* from a duck (Turkestan), and in 1884, *H. inflexa* from a chicken, but as the number and arrangement of the caudal papillae in his figure coincide with those of *A. lineata*, he probably was dealing with the latter as was also Lane (1914) who described *A. hamia* from chickens in India. Boulenger (1923), reporting *A. lineata* from Zanzibar, likewise considers *A. hamia* Lane a synonym of *A. lineata*. Another African record of this species is that of Gedoelst (1916) from the Belgian Congo. The Asiatic records of *A. lineata* include the Philippine Islands and Indo-China by Schwartz. This parasite has been reported recently from Canada by Baker, *et al.* (1929) who found it frequently at Montreal. In the British Isles, Lewis (1930) reported it from Wales, and I have found it in 8 out of 80 chickens examined at Cambridge this year.

While the name *Ascaris galli* was given by Schrank in 1788, he did not describe the parasite, but based the species on Goeze's (1782) composite species, *Ascaris teres*, a nematode Goeze had found in dogs, cats, chickens, and raptores. Freeborn (1923), substituting *galli* for *perspicillum*, without characterising the species, writes "...vermifuges for the intestinal worms of poultry, *Ascaridia galli* Schrank 1788 (= *A. perspicillum*)." Cram (1927), who degrades *A. perspicillum* to a synonym of *A. galli*, separates the latter from *A. lineata* on the basis of the larger size of the pre-anal sucker (over 217μ) and spicules (over 1·9 mm. long) and of the prominence of the lateral alae (very slender). Specimens of *A. lineata* in the present studies had pre-anal suckers 280μ in diameter, spicules 2·4 mm. long, and conspicuous lateral lines.

Whether a species of large nematode, besides *A. lineata* (Schneider), occurs in the small intestine of chickens is uncertain. If it be found that two instead of three pairs of sub-terminal caudal papillae and lateral alae occur constantly in such nematodes, there doubtless would be a second species. In the hope of settling this matter I examined *Ascaridia* material in the British Museum[1] from Uganda, Tangier, Gold Coast, Madras and (?) Germany, with the result, however, that no departure from the characters of *A. lineata* were observed.

MATERIAL AND METHODS.

The material for this work consisted of chicken *Ascaridia* from Manhattan, Kansas, U.S.A., and from Cambridge, England, as follows: from Kansas, 170 of the mature nematodes which were measured, many male specimens from other experiments examined only for number and distribution of caudal

[1] I wish to thank Dr H. A. Baylis for permitting me to examine the *Ascaridia* material in the British Museum (Natural History) and for his valuable advice.

papillae, and numerous immature worms from chickens (*Gallus domesticus* Linn.) reared in confinement and subsequently parasitised; from Cambridge, 50 mature *Ascaridia* from chickens brought to the markets, and many young and adult specimens from chickens raised parasite-free and later fed with embryonated eggs of the nematode.

The drawings and measurements were made both from live specimens and from those fixed in 5 per cent. formalin in distilled water. Dissection was accomplished most readily with fresh, live worms. Details of the cloaca and related organs were determined from transverse and longitudinal sections of the nematode fixed in Bouin's fluid and stained with haematoxylin and eosin. To obviate mistaking internal structures for papillae, the nematodes were pinned at various angles to the margin of a circular opening in the centre of a cork disc fitted into a Petri dish. This permitted the use of direct and reflected light on the immersed specimen and of low and high-power objectives. All drawings were made with the aid of a camera lucida.

Description.

Ascaridia lineata (Schneider 1866) Railliet and Henry 1912.

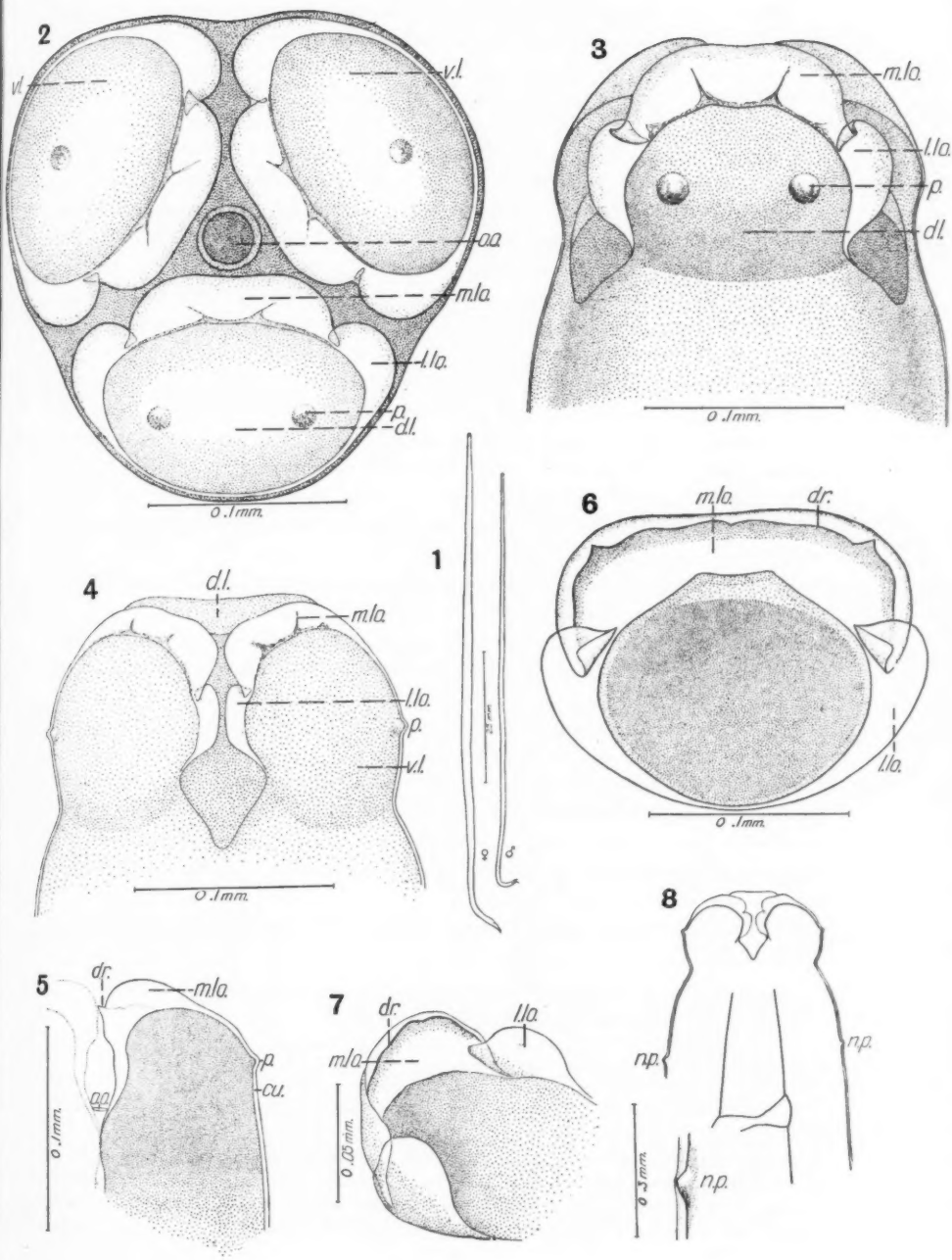
Synonymy: *A. hamia* Lane 1914.
? *A. braziliensis* Magalhães 1892.

The male and female *A. lineata* (Text-fig. 1, p. 363) are semi-transparent nematodes of medium size. The oral opening (Text-figs. 2, 5, *o.o.*) is surrounded by three prominent lips, one dorsal and two sub-ventral, each of whose distal margins is divided into three lobes; one median and two lateral (Text-figs. 2–7, *m.lo.*, *l.lo.*). The inner face of the median lobe bears a dentigerous ridge (Text-figs. 5–7, *d.r.*) in such position as to enable the nematode with its powerful lips to lacerate the intestinal epithelium, even though the opposing dentigerous ridges are not parallel (Text-fig. 2). Two conspicuous papillae occur on the dorsal lip and one on each of the sub-ventral lips (Text-figs. 2–5, *p.*). Schwartz reports two papillae on each sub-ventral lip of *A. lineata* as does also Lane (1914) for *A. hamia* which is probably identical with *A. lineata*. So-called neck papillae (Text-fig. 8, *n.p.*) occur occasionally, but not constantly, at the sides of the body near the anterior end.

Explanation of Text-figs. 1–8. *Ascaridia lineata* ♂ ♀, anterior end.

1. Adult ♂ and ♀, proportionate size.
2. Oral view of anterior end.
3. Dorsal view of anterior end.
4. Ventral view of anterior end.
5. Longitudinal section through lip and oral papilla.
6, 7. Medial views of lips.
8. Ventral view of anterior end showing neck papillae of rare occurrence.

Key to lettering: *cu.* cuticle; *d.l.* dorsal lip; *d.r.* dentigerous ridge; *l.lo.* lateral lobe; *m.lo.* median lobe; *n.p.* neck papilla; *o.o.* oral opening; *p.* papilla; *v.l.* sub-ventral lip.


2
v.l.
v.l.
oa.
m.lo.
l.lo.
p.
dl.
0.1mm.
3
m.lo.
l.lo.
p.
dl.
0.1mm.
1
♀
♂
6
m.lo.
dr.
l.lo.
0.1mm.
4
dl.
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l.lo.
p.
v.l.
0.1mm.
5
dr.
m.lo.
p.
cu.
ap.
0.1mm.
7
dr.
l.lo.
m.lo.
0.05mm.
8
n.p.
n.p.
n.p.
0.3mm.

Males. The males, slightly smaller and more slender than the females (Text-fig. 1), are 5·1–7·6 cm. long (average 6·3 cm.). Width: at anterior end 0·28–0·41 mm. (av. 0·37 mm.), at middle of body 0·49–1·21 mm. (av. 0·93 mm.). Oesophagus 2·3–7·2 mm. long (av. 3·5 mm.), width at posterior end 0·21–0·66 mm. (av. 0·38 mm.). Nerve ring from anterior end 0·3–1·46 mm. (av. 0·67 mm.) (Text-fig. 9, *n.r.*). Excretory pore from anterior end 0·93–1·29 mm. (av. 1·13 mm.). Pre-anal sucker varying in outline from oval to circular; longitudinal diameter 0·16–0·28 mm. (av. 0·23 mm.), transverse diameter 0·16–0·26 mm. (av. 0·23 mm.). This strongly developed sucker which obviously is of great importance in copulation bears in its posterior margin a minute ring (Text-fig. 10, *su.w.*). The anus, a transverse slit in a distinct prominence (Text-fig. 11, *an.*), lies 0·48–0·85 mm. from the tip of the tail (av. 0·73 mm.).

The caudal papillae (Text-figs. 10, 11, *pr.an.p.*, *an.p.*, *po.an.p.*, and *s.t.p.*), prominent among the taxonomic characters, occur in four groups: (1) pre-anal, three pairs, (2) anal, one pair, (3) post-anal, three pairs, and (4) sub-terminal, three pairs. Of the pre-anal papillae, the second pair lie close to the median lateral walls of the pre-anal sucker, the first pair distinctly anterior to the sucker, and the third pair about half-way between the sucker and the anus. The anal papillae (Text-figs. 10, 11, *an.p.*), the largest and most prominent, lie adjacent to the anus, their points piercing the caudal alae where broadest. Of the post-anal papillae, the first and third pairs are lateral, while the second pair which are smaller and lie close to the first pair are more medially placed and readily classed as ventral in position. The first pair of the sub-terminal group of papillae (Text-figs. 10, 11, *s.t.p.*) are the smallest of the ten pairs of caudal papillae and are the only ones whose distal points do not protrude regularly beyond the alae or caudal cuticle. Much variation in position occurs in the last two pairs of the sub-terminal group of papillae. One pair are distinctly lateral, the other, ventral. Absence of a papilla as shown by Schwartz (p. 767, Fig. 9), has been noted occasionally as has also the presence of supernumerary (twin) caudal papillae. In this connection it should be noted that Schwartz's reproduction (p. 766) of *A. hamia* from Lane (p. 663) erroneously omitted one of the caudal papillae.

The well-developed spicules are of about equal size and 1–2·4 mm. long

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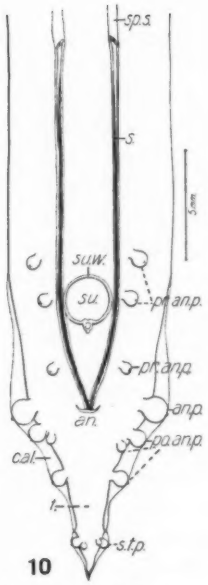
Explanation of Text-figs. 9–15. *A. lineata* ♂, digestive and reproductive systems.

9. Adult ♂, showing digestive and reproductive systems.
10. Ventral view of posterior end of ♂.
11. Composite side view of posterior end of ♂.
12, 13. Distal ends of spicules showing variable forms.
14. Proximal ends of spicules showing attachment of retractor muscles, and spicule sheaths.
15. Reproductive system of ♂.

Key to lettering: *an.* anus; *an.p.* anal papilla; *c.al.* caudal ala; *cl.* cloaca; *ej.* ejaculatory duct; *gl.* cement gland; *i.* intestine; *l.* lip; *n.r.* nerve ring; *oe.* oesophagus; *po.an.p.* post-anal papillae; *pr.an.p.* pre-anal papillae; *r.* rectum; *r.m.sp.* retractor spicule muscle; *s.t.p.* sub-terminal papillae; *sp.* spicule; *sp.s.* spicule sheath; *su.* sucker; *su.w.* sucker wall; *s.v.* seminal vesicle *t.* tail; *te.* testis.



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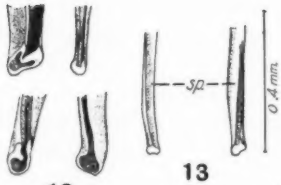

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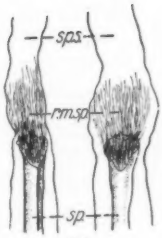

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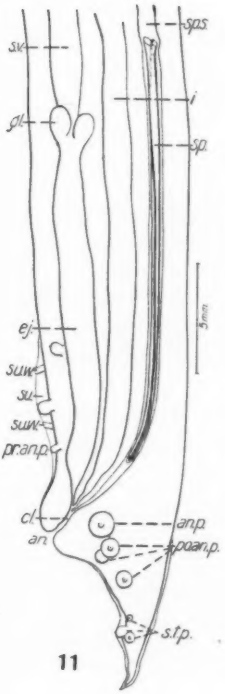

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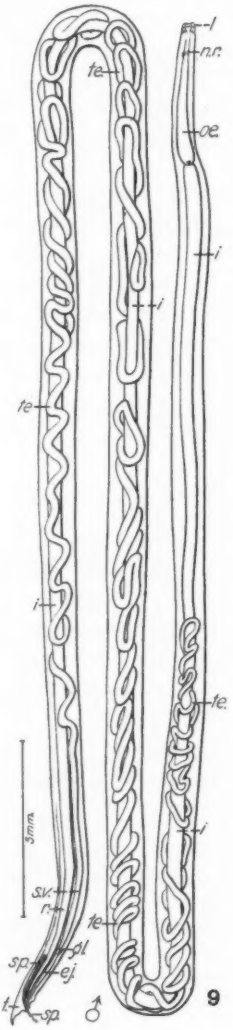

9

15

(av. 1·94 mm.). Their distal truncated ends vary widely in shape (Text-figs. 12, 13). Each spicule is enclosed in a separate sheath (Text-figs. 10, 11, 14, 15, *sp.s.*) and is withdrawn by an individual group of muscles attached to the proximal end of the spicule (Text-fig. 14, *r.m.sp.*). On being extruded, the two spicules pass from the distal ends of the sheaths through the dorsal portion of the cloaca and out of the anus. Other portions of the male genitalia include the long, single, irregularly coiled testis (Text-figs. 9, 15, *te.*) beginning in the anterior portion of the body, a somewhat thickened portion, the seminal vesicle (Text-figs. 11, 15, *s.v.*), filled with sperm cells in different stages, and a cylindrical ejaculatory duct (Text-figs. 11, 15, *ej.*), usually empty, opening into the cloaca. At the junction of the seminal vesicle and the ejaculatory duct are the so-called cement glands (Text-figs. 11, 15, *gl.*) which open into the ejaculatory duct. The simple digestive tract consists of the mouth, oesophagus which gradually widens posteriorly, and the long free intestine opening into the short cloaca.

Females. The females (Text-fig. 16), rather large and vigorous, are 7·2–11·6 cm. long (av. 8·8 cm.). Width: at anterior end 0·38–0·47 mm. (av. 0·42 mm.); of body at vulva 0·9–1·8 mm. (av. 1·3 mm.), of cuticular striations (Text-fig. 18, *cu.st.*) at middle of body 100μ. Oesophagus 2·1–5·1 mm. long (av. 3·9 mm.); width, posterior end, 0·36–1·57 mm. Nerve ring from anterior end 0·25–1·5 mm. (av. 0·75 mm.). Excretory pore from cephalic end 0·74–1·53 mm. (av. 1·3 mm.). Anus from tip of tail 1·31–1·88 mm. (av. 1·56 mm.) (Text-fig. 16, *t.*). Vulva from anterior end 3·1–5·4 cm. (av. 4·6 cm.). Eggs, 73–88μ × 45–50μ (av. 76 × 49μ).

Reproduction.

The female genitalia (Text-figs. 16, 17) consist of two extremely long, thread-like ovaries, one in the anterior, the other in the posterior portion of the body, two oviducts near the middle of the body, two uteri, one in the anterior, the other in the posterior portion of the body, and one central vagina (Text-fig. 18, *va.*) divided into a proximal portion with walls similar to those of the adjacent uteri, and a distal muscular portion that terminates in the vulva (Text-fig. 18, *v.*).

Eggs. The immature eggs arising from the walls of the anterior ovary (Text-figs. 16, 17, *o′.*) pass posteriorly through the lumen of the ovary to the

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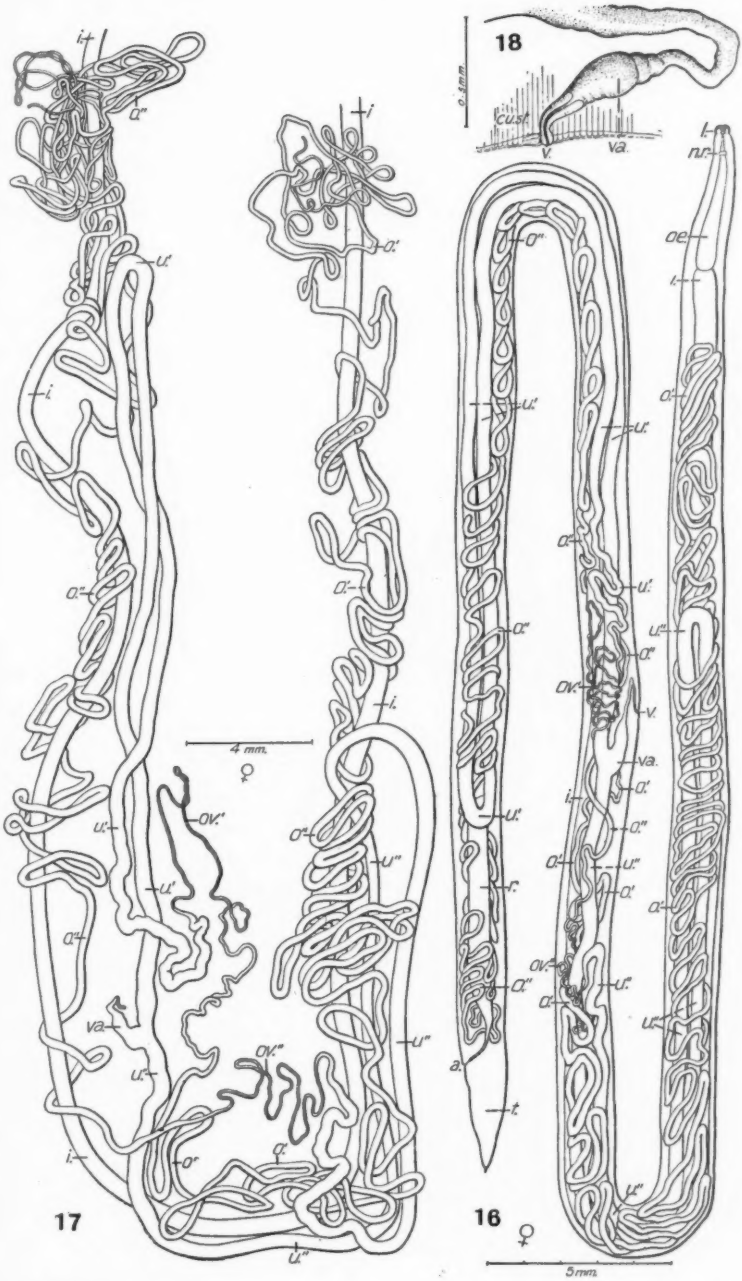
Explanation of Text-figs. 16–18. *A. lineata* ♀, digestive and reproductive systems.

16. Adult ♀, side view, showing digestive and reproductive systems.
17. Reproductive system of ♀.
18. Section body wall, showing cuticular striations, vagina and vulva.

Key to lettering: *a.* anus; *cu.st.* cuticular striation; *i.* intestine; *l.* lip; *n.r.* nerve ring; *o′.* anterior ovary; *o″.* posterior ovary; *oe.* oesophagus; *ov′.* oviduct connecting with anterior ovary; *ov″.* oviduct connecting with posterior ovary; *t.* tail; *u″.* anterior uterus; *u′.* posterior uterus; *v.* vulva; *va.* vagina.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corresponding narrow oviduct (*ov'*.) immediately posterior to the position of the vulva (Text-fig. 16, *v*.). The maturing eggs continue through the small, thin-walled oviduct into the gradually widening posterior uterus (Text-figs. 16, 17, *u'*.), where they may be met by ascending spermatozoa and fertilised. Continuing on in the looped posterior uterus, the egg receives its shell (Pl. XIII, fig. 1), being eventually extruded through the vagina and vulva. Eggs arising in the posterior ovary (Text-figs. 16, 17, *o''*.) pass anteriorly, arriving at the oviduct (*ov.''*) situated somewhat in front of the vagina (*va.*) and entering the anterior uterus (Text-figs. 16, 17, *u.''*). Whether fertilised or not, they pass slowly onward in the packed, looped uterus. In young females, the nearer the eggs are to the proximal end of the uterus when the shell is acquired, the better is the chance of fertilisation. Sampling the anterior uterus of a live *A. lineata* at six levels and counting 100 eggs at each level the following percentages of fertilisation were found: (1) at a point in the distal (narrow) part of the uterus, 0 per cent.; (2) at 10 mm. nearer the vagina, 3 per cent.; (3) at 20 mm. nearer, 65 per cent.; (4) at 30 mm. nearer, 90 per cent.; (5) at 40 mm. nearer, 98 per cent.; and (6) at 45 mm., or at about the level of the vagina, 98 per cent. of fertilisation.

In the fully matured females shells begin to form on eggs near the distal end of the uterus. Only in the first few millimetres of the uterus (distal end) are shell-less eggs to be found in adult females. In this portion occur sperm cells in an advanced stage of development. The eggs when laid (Pl. XIII, figs. 1, 2) are covered with three envelopes: (1) an inner, highly impermeable one, the vitelline membrane, (2) the thick resistant shell, and (3) a thin, albuminous covering. In one end of the egg is a structure bearing some resemblance to a micropyle, but which, with the aid of a Chambers' double micromanipulator[1] proved to be a solid conical appendage of the vitelline membrane, free from the shell. This structure occurs in the genus *Ascaridia*, according to Baylis (1929) who considered it to be an internal thickening of the shell. Dorman (1928), who describes it as an opercular plug, thought it had a definite rôle in hatching of the larvae of the caecal worm, *Heterakis papillosa* (Bloch).

Incubation. When the eggs are deposited, they are either unfertilised (Pl. XIII, fig. 1), the cytoplasm being of approximately even density, or fertilised (Pl. XIII, fig. 2), the rather clear equatorial spot facilitating the identification of eggs capable of development. Under conditions affording a suitable supply of oxygen, moisture and heat, the fertilised egg divides into the two-cell stage (Pl. XIII, fig. 3), the rate of division depending chiefly upon the temperature and oxygen supply. When immersed in distilled water to a depth of 3 mm. and kept at about 30° C., the time from one cleavage stage to the next is about 12 hours. That either of the two blastomeres may divide first is shown in Pl. XIII, figs. 5 and 7. Similarly on approaching the four-cell stage (Pl. XIII, fig. 9), the order of the dividing cells is inconstant (Pl. XIII, figs. 6, 8). In about 3 days,

[1] I wish to thank Dr Robert C. Chambers for his personal assistance in determining the nature and the relationship of this structure to the egg envelopes.

cleavage progresses to the morula with large blastomeres (Pl. XIII, fig. 17) after which more divisions occur simultaneously and the morula with small blastomeres (Pl. XIII, figs. 21, 22) is reached in about 5 days. As development proceeds the cells become smaller, and those at one end of the embryo, less opaque (Pl. XIII, fig. 23). Between the more and the less granular cells, a line of separation appears (Pl. XIII, fig. 24), and in about 3 days there is developed the "tadpole" stage (Pl. XIII, figs. 26, 27) with blunt anterior and conical posterior ends. Two additional days of incubation result in the development of a vermiform embryo (Pl. XIII, figs. 29, 30). This is followed by continued increase in length and decrease in width, and by further reduced terminal opacity (Pl. XIII, figs. 31–35). Such embryos, while beginning to coil, cannot maintain themselves in the host. Among the changes occurring as the embryos become infective (Pl. XIII, figs. 36–40) are clearer differentiation of the digestive tract, formation of a dorsal oral prominence, more pointed extremities, and further increase in length which necessitates more coiling of the embryo.

Given suitable conditions of moisture and temperature (*v. supra*) development to the infective stage may occur in about 16 days. By raising the temperature to 33° C., and decreasing the water of the culture to a depth of 1 mm., a large percentage of the fertile eggs will become infective in 10–12 days. Temperatures approaching the optimum (30–33° C.) are an important factor in evenness of development as similar cultures kept at room temperatures develop very irregularly, some eggs remaining in the early cleavage stages while others are morulae with small blastomeres.

Viability of eggs. Studies made on the extremes of temperature endured by the eggs of *A. lineata* have shown that in the early cleavage stages, the eggs survived freezing at − 12° to − 8° C. for 15 hours, but not 22 hours. Twelve hours' exposure to 43° C. proved to be lethal for eggs in all stages of development. Applying higher temperatures Itagaki (1927) found that exposure of *A. perspicillum* eggs to 50°–53° C. for 5 minutes was not lethal, but that 54° C. for this period killed all eggs. Exposing eggs of *A. lineata* for longer periods, I found that fertile eggs kept 1 month at 0° C. were unable to reach the infective stage when incubated at 30° C., whereas, eggs from the same cultures kept concurrently at 10° C. for a month developed normally to the infective stage when incubated at 30° C. While the month's exposure of the eggs to 0° C. did not kill them, it greatly retarded the cleavage which began after the eggs were exposed to the higher temperature. Few eggs progressed beyond the four-celled stage, the morulae and all others disintegrating in 3 weeks. The temperature at which cleavage begins in water cultures of *A. lineata* is between 10° and 15° C.[1] As eggs kept in a chest at laboratory temperatures (20°–24° C.) have remained viable for 6 months, it is evident that moderate temperatures are more conducible to longevity of the eggs than those approximating 0° C. which are so effective in preserving animal tissues. Results of a

[1] Acknowledgment is made for use of refrigeration rooms in the Low Temperature Research Station, University of Cambridge.

series of tests on the viability of *A. lineata* eggs exposed to natural conditions in soil and carried on during the past decade will appear in a separate paper.

Development in the host.

Hatching of the eggs. While the eggs normally hatch in the fowl's duodenum, they may hatch in water cultures. The observations here recorded were made on eggs in cultures. In hatching, the mature coiled embryo protrudes its anterior end through an opening in the shell (Pl. XIII, fig. 41), moving on out, apparently by virtue of bracing its posterior portion against the shell walls. If the opening be small, there are several rest periods before half of the body has emerged, when, with snake-like movements, the exit is soon completed. How the hatching orifice is made is undetermined. Mature embryos within the egg show a distinct dorsal, oral prominence (Pl. XIII, figs. 43, 44, and Text-fig. 21). Although this structure is pressed against the vitelline membrane and

Table I. *Showing results of attempts to infest chickens by feeding* A. lineata *larvae.*

Fowl no.	Age of fowl in days when fed	No. of larvae fed	Source of larvae	Age of fowl in days when killed	No. of *A. lineata* found in intestine	Remarks
351	139	500	Egg culture	179	0	—
352	139	500	,,	182	0	—
359	30	100	,,	36	0	—
362	31	61	,,	77	2	Adult ♂'s
364	34	60	,,	82	1	Adult ♂
365	34	100	,,	82	1	,,
970	20	50	Fowl's gut	34	3	Larvae given in water
971	20	50	,,	37	2	,,
979	21	50	,,	34	0	—
994	34	800	,,	52	0	Larvae given in mash
995	40	400	,,	55	0	,,

inner wall of the shell repeatedly, there is no certainty that it functions as a hatching spine. "Epidemics" of hatching occur in some egg cultures, but not in others. As pressure on the egg readily ruptures the shell and enables the larva to escape, Baker, *et al.* suggest that it may be one of the hatching factors. Artificial digestive juices and incubation at temperatures up to 37° C. have failed to produce hatching as has also the sulphuric acid-salt solution in which a few *H. papillosa* hatched (Dorman).

Infestations from free larvae. The hatching of *A. lineata* eggs in water cultures indicates that such eggs would hatch to some extent in moist soil. Could such larvae infest chickens feeding on soil? To test this, free larvae, either from water cultures of eggs, or recovered from infested chickens were given to chickens raised parasite-free in confinement. The results of the tests are given in Table I. As an example, Fowl 359, when 30 days old, was given with a pipette 100 live *A. lineata* larvae (many active) which had hatched in a water culture. Six days later, the bird was killed and examined, but no

larvae were found. When this bird was given the free larvae, Fowl 358 was fed 200 embryonated eggs of the nematode for comparison. Five days later this bird was killed and examined; many active larvae were found in its small intestine. Further examination of Table I shows that, occasionally, a few free larvae were able to establish themselves in the fowl's intestine. Larvae recovered from the intestines of chickens were always more vigorous, but they were scarcely more infective (Fowls 970–995) than were those that hatched in the egg cultures (Fowls 351–365). In the case of Fowls 970 and 971, both of which became infested, the larvae were administered with a pipette in water which probably facilitated their passage through the gizzard. The larvae fed to Fowls 979 and 994 were equally vigorous, but their administration in a moist mash, approximating natural conditions, gave negative results, as did also the test on Fowl 995 to which larvae 2 weeks old were given. The results of these tests indicate that while infestation with *A. lineata* may follow when chickens swallow free larvae, this mode of infestation is rare in nature.

Infestations from embryonated eggs. That infestations of chickens with *A. lineata* are due generally to the ingestion of embryonated eggs is indicated by the results of tests by Ackert (1920, 1923), by Guberlet (1924), and by the findings in a series of experiments by Ackert and Herrick (1928). In one of these experiments, chickens 25 days old were given, individually, 500 embryonated eggs of *A. lineata*. Examinations of the birds within 3 weeks showed that only 2 of the 50 chickens were without worms. The infestations ranged from 1 to 394 young *A. lineata* with an average of 34 per bird. Of 14 chickens given 500 embryonated eggs by these authors in another experiment, 12 became infested, the range being from 2 to 65 larvae with an average of 15 per bird. From the results of these experiments and of those by Guberlet who parasitised chickens by giving embryonated eggs, but failed to do so by feeding larvae, it is evident that, in general, infestations of fowls with *A. lineata* are due to the ingestion of eggs rather than larvae.

Habitat and its hydrogen-ion concentration. The embryonated eggs of *A. lineata* when swallowed by the chicken may hatch in either the proventriculus or the duodenum according to Itagaki who speaks of the latter as the "muscular stomach." In determining the habitat of *A. lineata*, I have found that the larvae tend to accumulate in the duodenum a few centimetres behind the entrance of the bile ducts. In this habitat, the hydrogen-ion concentration of 56 chickens of various ages varied from 5·7 to 7·5 (av. of 6·7). Six chickens, starved for 24 hours, had a range of 6·2–6·6 (av. 6·4), while 6 control chickens from the same hatch and not starved had a range of 5·7–6·9 (av. 6·5). Among the cases in which worms were present in the habitat where the sample was taken were the following: 6·9 numerous larvae; 6·5, 20 *A. lineata*; 6·6, 1 nearly mature female; and 7·1, 17 young worms. The hydrogen-ion concentration of the duodenal loop anterior to the entrance of the bile ducts averaged 6·5, the intestinal lumen at the yolk sac diverticulum which is posterior to the normal habitat averaged 6·9, and the posterior part of the small

intestine (adjacent to the distal ends of the caeca), where healthy *A. lineata* are never found, averaged 7·3. While the worms do not appear to be markedly sensitive to weak acids or bases, they live in media that are not far from neutral.

Penetration of the intestinal mucosa. During the first 9 days after hatching the larvae live in the lumen of the posterior portion of the duodenum and in the intervillar spaces. On about the 10th day, the young worms begin to move deeply between the villi and to penetrate the intestinal mucosa (Pl. XIV, fig. 1) destroying Lieberkühn's glands and causing haemorrhages (Ackert, 1923). From the 10th to the 17th day after feeding embryonated eggs of *A. lineata*, young worms with their anterior ends buried in the mucosa and with the posterior half projecting into the lumen are readily found. Ordinarily, by the 18th day, the larvae have withdrawn from the mucosa and are to be found free in the lumen of the posterior portion of the duodenum—the usual habitat of *A. lineata* when 3 weeks old. From this time on my findings and those of my associates indicate that the worms live free in the intestinal lumen. Itagaki (1927, 1930) describes nodules, the size of a pinhead or smaller, in the muscular wall of the intestines of chickens which he attributes to larvae of *A. perspicillum* (a nematode very closely related, if not identical with *A. lineata*). He states (1927, p. 340) that the minimum period required for the formation of the nodules is 36 days, and adds that they were usually found in the lower third of the small intestine. As this is not the habitat of *A. lineata* and as Itagaki found that the nematode may mature in 37 days, it seems unlikely that the tiny nodules were due to this nematode. Searches for such nodules in the small intestines of chickens, known to be free from other parasites, have failed to reveal them (both in Kansas and Cambridgeshire).

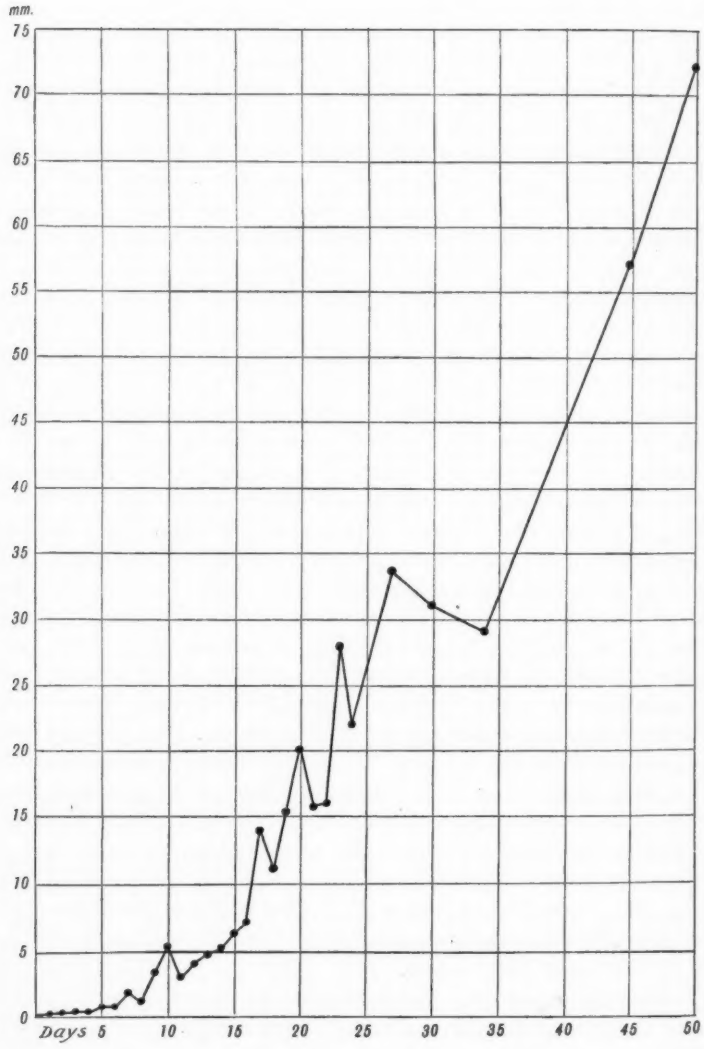
Migration of larvae rare. Tests on possible migration of *A. lineata* larvae outside of the intestines of chickens made by Ackert (1920, 1923), and by Guberlet showed that, occasionally, a larva was found in some part of the body other than the intestine. Such tests, to the number of 74, have now been carried out by me. In every case several hundred embryonated eggs of the nematode were fed to the fowls which were killed and autopsied after various intervals of time ranging from 1 to 14 days. In all cases there were larvae in the small intestine; and in some cases when no larvae were found in the liver or other organs, the intestines contained nearly 2000 of the young nematodes. None were taken from the kidneys, spleen or body cavity. In only 6 birds (designated by the letters *a–f*) were a few larvae encountered in other organs as follows: (*a*) 2 from the liver, (*b–e*) 1–4 from the lungs, and (*f*) 1 from the trachea. These results agree closely with those of Guberlet, who, in many tests, seldom found larvae outside of the intestine. His experiments yielded the following numbers of larvae from other sources than the intestine: lungs, 6, 2; trachea, 1, 1; kidney, 1; spleen, 1, 1, 1; and body cavity, 2. These experiments show that migration of *A. lineata* larvae outside of the fowl's intestine is unusual. To determine the probable route taken by the few migrating larvae, transverse sections were made of infested intestines. In Pl. XIV are shown

sections of larvae which indicate that the route was from the lumen through Lieberkühn's glands (Pl. XIV, fig. 1), the muscular layers (Pl. XIV, figs. 2, 3), to the mesenteric folds (Pl. XIV, fig. 4). The comparatively large size of the larvae makes a vascular migratory course highly improbable.

Rate of growth. The rate of growth in length of young *A. lineata* depends upon the age of the host (Ackert and Herrick, 1928; Herrick, 1926), upon the nutrition of the host (Ackert, McIlvaine and Crawford, 1931; Ackert and Nolf, 1931), and probably upon other factors including heritance. An idea of the rate of growth of *A. lineata* in young chickens may be obtained from Text-fig. 19 which represents the growth in length of 293 of the young nematodes from 73 birds examined over a period of 50 days. The birds were about a month old when parasitised. Starting with the newly hatched larvae *ca.* 0·3 mm. long (Text-fig. 19) the growth is gradual up to about the 8th day when the length is *ca.* 1 mm. During the second 8-day period growth is much more rapid, the young worm at 16 days of age averaging 7 mm. in length. A further increase in rate of growth is noted in the third period of 8 days when the larvae average 22 mm. in length. During the fourth and fifth periods, the growth is somewhat slower, the respective lengths attained being 30·5 mm. and 44 mm. In the sixth period, the nematodes averaged 66 mm., and in 2 days more there appeared mature females and males. Averaging the growth by periods of 8 days, it was found that the larvae grew about 0·12 mm. per day during the first period, 0·75 mm. in the second period, 1·5 mm. during the third, fourth, and fifth periods, and 3 mm. per day during the sixth period. With larger numbers of individuals the Graph (Text-fig. 19) doubtless would have been modified to some extent. This growth rate is comparable to that found by Kempster (1926) for chickens (white rocks, p. 112), being gradual at first, faster toward the middle of the period, and rapid as maturity approaches.

Moulting and development of taxonomic characters. To study the general development of the young *A. lineata*, 30 chickens 1 month old were parasitised and one bird examined each day. Much variation in size and development occurred, but the comparisons of body form, especially that of the anterior and posterior ends of the worms made it possible to determine that three moults occurred before the adult stage was reached. In moulting, the old cuticle splits anteriorly, whereupon the anterior end of the larva is freed (Text-fig. 20). The lashing movements of the larva slowly crumple the sheath which, on further splitting, is lost.

The newly hatched thick-bodied larva (Text-fig. 21, p. 376) has a long slender rhabditiform oesophagus and an intestine with indistinct cellular wall. One day after hatching, the lateral folds (Pl. XIV, fig. 1, *l.f.*), extending from the oesophagus nearly to the tail, are present as is also the small ovoid reproductive anlage which is situated ventrally somewhat posterior to the middle of the body (Text-fig. 21, *r.a.*). Among the changes of the first 3 to 5 days are a narrowing of the anterior oesophageal swelling, a much more darkened and cellularly distinct intestine and a more slender body. Changes during the 6th to



Text-fig. 19. Graph showing rate of growth in length of 293 *A. lineata* obtained from 73 chickens killed and examined during a period of 50 days; the birds about 1 month old when parasitised.

8th day, when the first moult occurred, include the presence of the nerve ring, and characteristic changes in the tail which becomes somewhat sickle-shaped (Figs. 22, 23) and shows an anal prominence. The males can now be distinguished by the presence of a pre-anal swelling (Fig. 23), the beginning of the pre-anal sucker. Measurements of 8 of these larvae gave the following proportions: length 4·2 mm.; width, middle of body 0·08 mm.; width, dorso-ventral at anus 0·07 mm.; anus from tip of tail 0·2 mm.; nerve ring from anterior end 0·13 mm.; oesophagus 0·39 mm. long by 0·03 mm. wide at bulb.

By the 11th to the 13th day, the pressure of the developing pre-anal sucker against the adjacent cuticle gave evidence of the approaching second moult which occurred in different larvae on the 14th and 15th days. The principal changes common to both sexes were: lips with oral papillae and dentigerous ridges and the substitution of lateral lines even with the surface of the body for the projecting lateral folds; in the females, a proportionately shorter tail (Fig. 24), presence of vulva and short vagina; males with prominent anus and pre-anal sucker, and with three pairs of papillae: one pre-anal pair, one pair immediately behind the anus, and the other pair about mid-way between the anus and the tip of the tail (Fig. 25). Several females on the 14th day averaged 5·9 mm. × 0·12 mm. Males on the 15th day averaged 6·4 mm. × 0·12 mm.

By the 18th day there was marked variation in development. Evidences of the third moult occurred daily from now until the 22nd day, when a specimen was found with the old cuticle distinctly separated from the new, which bore the papillae characteristic of the adult male. In external characters the young *A. lineata* now (after the third moult) closely resembled the adults. The lateral lines even with the surface of the body, lips with oral papillae and dentigerous ridges in both sexes, the slightly protruding lips of the vulva in the females and the pre-anal sucker and ten pairs of caudal papillae in the males, all of these characters were very similar to the corresponding ones in the adults. Measurements of a few young worms on the 19th day were: females 15·5 × 0·28 mm.; males 14·5 × 0·26 mm.; the spicules were well developed.

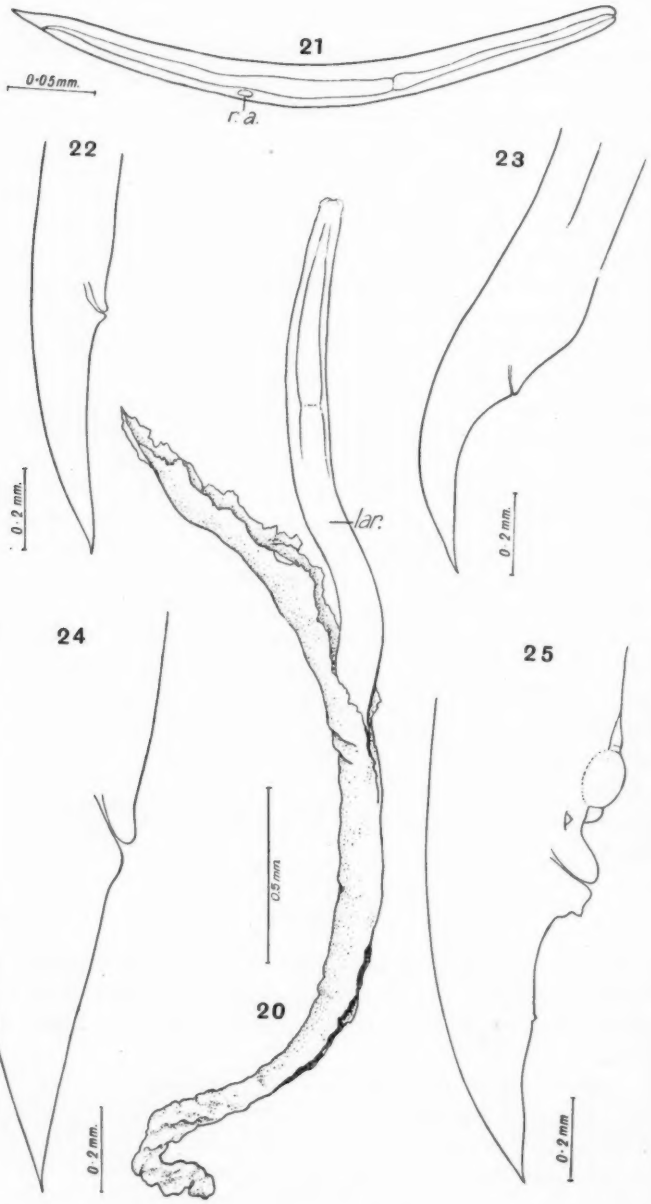
SUMMARY.

1. Studies were made on the morphology, with special emphasis on the taxonomic characters, of 220 mature male and female *Ascaridia lineata* (Schneider) from chickens at Manhattan, Kansas, U.S.A., and Cambridge, England.

2. Eggs arising in the anterior ovary pass into the posterior uterus; similarly, eggs from the posterior ovary pass into the anterior uterus.

3. In young females eggs may be fertilised at any place in the uteri; in adults, fertilisation occurs near the distal ends of the uteri.

4. With the aid of a micromanipulator, a small structure in one pole of the mature egg, previously described as an opening, an opercular plug in the shell,


21
0·05mm.
r.a.
22
0·2mm.
23
0·2mm.
lar.
24
0·2mm.
0·5mm
20
25
0·2mm

or as an internal thickening of the shell, was found to be a solid, conical appendage of the vitelline membrane, free from the shell.

5. Fertilised eggs develop to the coiled embryo (infective) stage in 16 days when incubated in water at 30° C.; in 1 mm. of water at 33° C., they become infective in 10 days.

6. Water cultures of fertile eggs do not develop when kept at constant temperatures of 0° C., or of 10° C.; at 15° C., development proceeds.

7. Constant refrigeration of fertile eggs in water cultures for 1 month at 0° C. so lowers the vitality that, on being incubated subsequently at 30° C., they divide slowly and soon die; keeping the eggs at 10° C. for 1 month has no deleterious effect on them when incubated subsequently at 30° C.

8. In hatching, the embryo may escape from any part of the egg shell, either in the duodenum of the host, or in water cultures; newly hatched larvae swallowed by the chicken seldom become established; infestations normally result from the ingestion of embryonated eggs.

9. The habitat of *A. lineata* is the duodenum, especially the portion which is a few centimetres posterior to the entrance of the bile ducts; determination of hydrogen-ion concentrations showed that the nematodes live in nearly neutral media (av. 6·7).

10. Penetration of the duodenal mucosa by larvae 10–17 days old is frequent in young chickens; occasionally, a larva goes on through the intestinal wall to the liver and lungs, but ordinarily, after the 17th day the young worms withdraw from the mucosa into the lumen of the intestine.

11. Young *A. lineata* grew to maturity in 50 days in chickens parasitised when about a month old; in six 8-day periods following hatching of the larvae, the average daily growth in length per period was about 0·12 mm. in the first period, 0·75 mm. in the second, 1·5 mm. in the third, fourth, and fifth periods, and 3 mm. in the sixth period.

12. At least three moults occur before the adult form of the nematode is developed. Important morphological changes associated with the moults include: *after first moult*, presence of pre-anal swelling (males), and of anal prominence in both sexes; *after second moult*, lips with oral papillae and dentigerous ridges, projecting lateral folds (alae) present in newly hatched larvae replaced by non-projecting lateral lines, females with vulva and shorter tail proportionately, and males with pre-anal sucker and three pairs of caudal papillae; *after third moult*, external characters similar to those of the adult *A. lineata*.

Explanation of Text-figs. 20–25. Young *A. lineata*.

20. Larva moulting. *lar.* larva.
21. Day-old larva, showing dorsal oral prominence. *r.a.* reproductive anlage.
22. Tail of larva (♀) after first moult.
23. Tail of larva (♂) after first moult.
24. Tail of larva (♀) after second moult.
25. Tail of larva (♂) after second moult.

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[1] In these references *Ascaridia perspicillum* (Rud.) refers to *Ascaridia lineata* (Schneider).

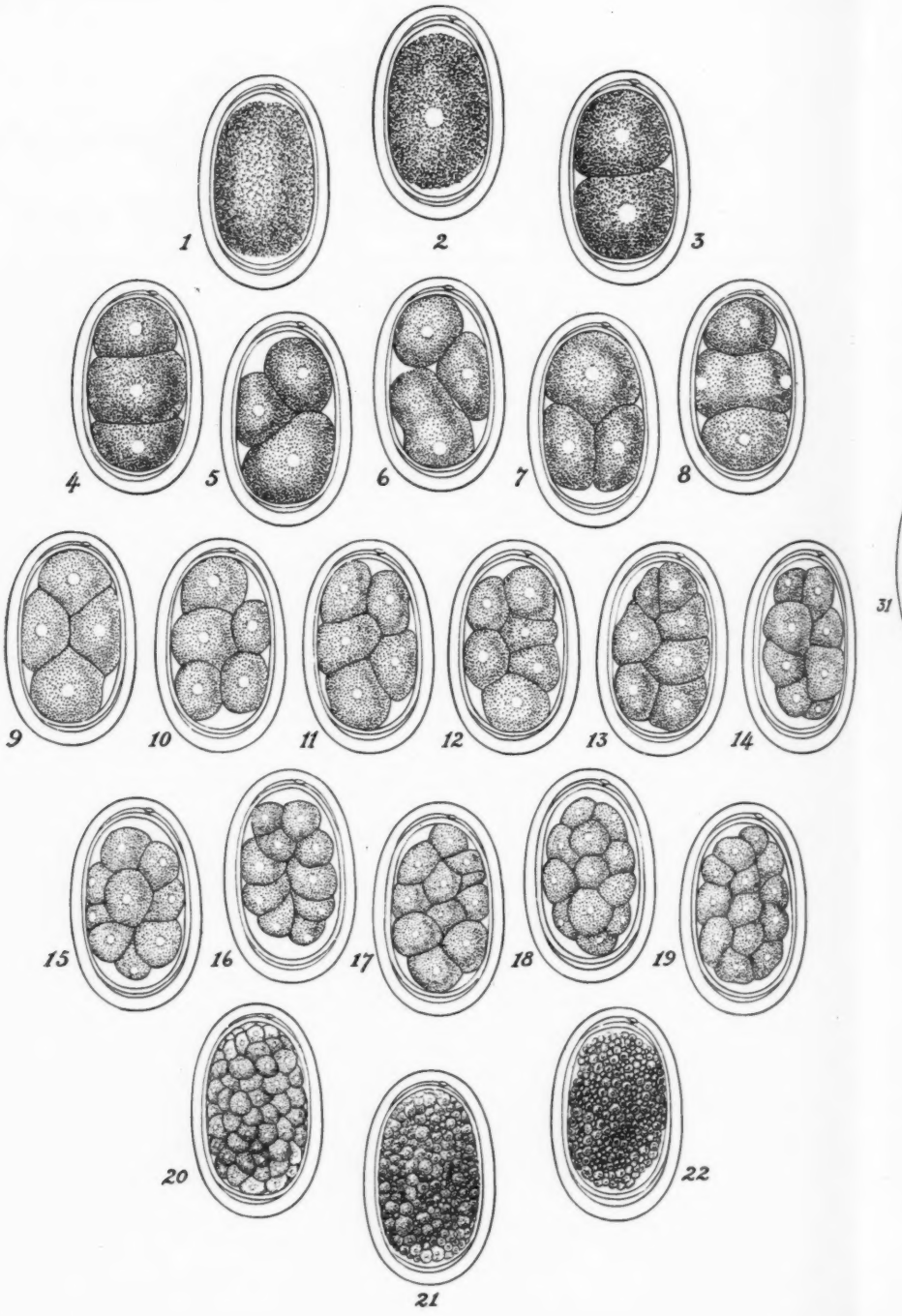

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PLATE XIII

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EXPLANATION OF PLATES XIII AND XIV.

PLATE XIII.

Figs. 1–46. *A. lineata*, eggs.

1. Mature infertile egg.
2. Fertile egg.

3–16. Eggs in cleavage stages.

17–20. Morulae with large blastomeres.

21, 22. Morulae with small blastomeres.

23–25. Initial stages of differentiation.

26, 27. "Tadpole" stages.

28–35. Early vermiform embryos.

36–40. Coiled embryos (embryonated eggs).

41–46. Stages in hatching.

PLATE XIV.

Figs. 1–4. Sections of *A. lineata* larvae and surrounding intestinal tissues. From photographs of transverse sections of chicken intestines.

1. Transverse section of larva destroying Lieberkühn's glands. *l.f.* lateral fold.
2. Oblique section of larva showing path of migration through muscular layers of intestine.
3. Transverse section of larva nearly through intestinal wall.
4. Longitudinal section of larva in mesenteric fold.

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THE LARVAL AND PUPAL ANATOMY OF *STENOMALUS MICANS* OL. (PTEROMALIDAE), A CHALCID ENDO-PARASITE OF THE GOUT-FLY OF BARLEY (*CHLOROPS TAENIOPUS* MEIG.), WITH SOME DETAILS OF THE LIFE HISTORY OF THE SUMMER GENERATION.

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(With 5 Figures in the Text.)

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Introduction.

Four species of Hymenopterous parasites of the gout-fly of barley have been found in certain of the southern counties of England.

	Endoparasites.	*Ectoparasites.*
Braconids.	*Coelineus niger* Rees, J.	*Bracon longicollis* Westmall. ?
Chalcids.	*Stenomalus micans* Ol.	*Stenomalus* spp. probably *laetus* Rusch.

Full details of the host (*Chlorops taeniopus*) are given in papers by Frew who also describes three types of damage caused to barley shoots by the gout-fly larvae: (*a*) Summer-type damage: well developed ear partially visible through sheathing leaf; (*b*) Winter-type damage: very stunted growth and shoot with no trace of ear; (*c*) Intermediate type of damage between types (*a*) and (*c*).

The "gouted" shoots showing winter type of damage were collected from mid-June until the third week in August of 1928, from the following localities: Wiltshire (Calne and Marlborough), Somerset (Peasedown nr. Radstock, English Combe nr. Bath, Tog Hill nr. Marshfield, and Taunton), Hampshire

(Basingstoke), Herts. (Harpenden), Surrey (Woodmansterne nr Epsom, and Reigate), Kent (Maidstone and Charing).

Over 10,000 "gouted" shoots were examined and in every locality mentioned the endoparasites were present in up to 68 per cent. of the "gouted" shoots picked. This percentage is based on a count of shoots of winter-type damage and does not include other forms of damaged shoots, which do not appear to be so heavily parasitised.

The spring of 1928 was very wet and caused late sowing of the barley in many districts and this was followed by slow growth of the tillers. These two factors together provided the most favourable conditions for the oviposition of the gout-flies, and the resulting infestation of the barley shoots was high, especially of the winter-type damage. An exception was found in Kent where the climatic conditions had been more favourable for the preparation of an earlier and better seed bed.

Most of the "gouted" shoots were collected from localities in Somerset and Wiltshire and, although the endoparasites were very common, not one ectoparasitic larva was found despite a most careful search. From a preliminary study and from data supplied by Dr J. G. H. Frew the ectoparasites were known to be present in the barley crops in previous seasons on Hoos Field, Rothamsted Research Station. Therefore some 3000 shoots (winter-type damage) were collected from different parts of that field. On examination, many gout-fly larvae were found to have pupated, or about to pupate, and only two ectoparasitic larvae (*Stenomalus* spp.) were found. The endoparasites were present in 42 per cent. of the "gouted" shoots.

Therefore it would seem that the ectoparasites in 1928 were absent from all districts examined, except Harpenden where they were very scarce. The probability that they were missed seems very slight as full precautions were taken to detect them; every gout-fly larva was examined for the mandible marks of the ectoparasites which are conspicuous on the parasitised host. The examination of summer-type samples of "gouted" barley from various districts gave negative results and Frew's data show that this type of shoot is not so heavily parasitised.

To sum up, it seems that only the endoparasites have been recorded as the principal parasites of the gout-fly and that these appear to be distributed in southern England. The ectoparasites, so far, have only been found at Harpenden.

The percentages of "gouted" shoots that were parasitised were ascertained from samples collected from Peasedown and Tog Hill at intervals over a period of 4 weeks (see Table I). The shoots (winter-type damage) were collected as far as possible from the same area of the field. The instar in which the parasite was found was recorded in each case (see Table II) and the figures obtained, although not providing critical data, give an indication of the rate of development of the parasites.

The figures (Table II) obtained from the examination of the "gouted"

barley shoots collected from Peasedown give the following indications of the time required for the development of *S. micans* larvae, of which there are five larval instars.

1928	June 28	An occasional first instar larva.
	July 5	Most larvae in first instar.
	„ 13	Most larvae in second instar.
	„ 27	Most larvae had pupated.

The larval life thus takes approximately 20–22 days.

Table I. *The parasitism of* Chlorops taeniopus.

Locality	Date (1928) of collection of "gouted" shoots	Number of "gouted" shoots examined	Number of hosts parasitised	Total % *Chlorops* parasitised	Relative frequency of *S. micans* No.	*S. micans* %	*C. niger* No.	*C. niger* %
Peasedown	June 28	300	Few	—	—	—	—	—
„	July 5	511	322	63	Majority	Majority	A few	A few
„	„ 7	490	312	64	255	81·7	57	18·3
„	„ 13	504	337	67·9	227	67·3	110	32·7
„	„ 27	501	326	65	213	65·3	113	34·7
Tog Hill	„ 24	472	283	60	204	72·2	79	27·8

Table II. *The relative frequency of each stage of development of the endoparasites encountered.*

Locality	Date (1928) of collection of "gouted" shoots	% of *S. micans* found as: First instar larva	Second instar larva	Third instar larva	Fourth and fifth instar larva	Pupa	% of *C. niger* found as: Early instar larva	Late instar larva	Pupa
Peasedown	June 28	Occasional first instar larva					Occasional early instar larva		
„	July 5	Many	Few	0	0	0	A few	0	0
„	„ 7	39·1	32·4	22·4	6·1	0	94·7	5·3	0
„	„ 13	24·2	39·3	27·2	9·3	0	68·8	31·2	0
„	„ 27	0	*5·1	—	44·3	50·6	—	*5·3	94·7
Tog Hill	„ 24	—	*7·8	—	63·6	28·6	—	—	Most

* Includes larva up to late third instar.

With reference to the life history of *S. micans* it may be stated briefly that there are two generations of host and parasite within the year. In April the gout-fly (Frew 1924) deposits eggs on young tillers of the barley plant and as a rule there is but one larva per tiller; the adult parasite appears shortly afterwards and usually deposits one egg per young larval host. The parasitised host is eventually killed and the gout-fly larvae which escape parasitism produce a second brood of imagoes in August. This second generation of flies oviposits mainly on young couch grass (*Agropyrum repens*); a second generation of the parasite follows them and oviposits in the young larvae. The host and parasite winter as larvae; the parasite does not feed actively until spring when the host is nearly full grown.

1. TECHNIQUE.

The larvae of *Chlorops* were dissected in normal saline. In most cases the head was cut off and the body contents were readily expelled by gentle pressure applied in a forward direction at the posterior end of the body, the viscera escaping at the cut end, the parasite, if present, also floating out.

The parasitic larvae in the early instars (prior to the development of the spiracles) will live in normal saline for 3–5 days, and provided that they float, moulting will occur. In this manner it was possible to determine the instars with certainty. It is interesting that the apneustic larval instars of the endoparasitic Braconid *Coelineus niger*, although living in apparently the same environment in the host as *S. micans*, do not live over 12–18 hours on the surface of normal saline solution. With the endoparasitic Chalcid the larva usually floats on the surface of the fluid, the cuticle being unwettable; if the larva is pushed below the surface the fluid does not come into contact with the cuticle for over 20 hours. With the Braconid larva, however, the cuticle is more easily wetted and frequently after 14 hours the dead or dying larva sinks beneath the surface of the fluid. The tracheal system was studied on living larvae, the best results being obtained by keeping just-hatched or just-moulted larvae on saline solution for 2 days. Berelese's fluid was a valuable aid provided that the specimen was examined before the fluid had completely penetrated. All measurements were made on freshly dissected specimens and not on saline specimens, as the latter moult three times with little or no increase in size.

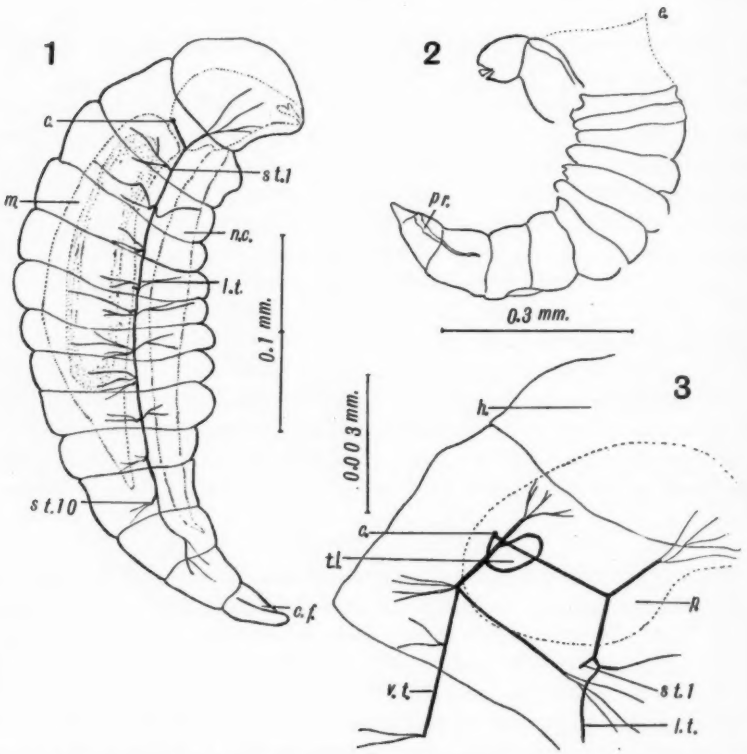
2. FIRST INSTAR LARVA.

The larva (Text-fig. I) has a characteristic semi-sickle shape. It has a large head and thirteen body segments, the posterior one in the form of a caudal furca. The cuticle is smooth and very delicate; setae are absent. Very young larvae are transparent or nearly so, a slightly opaque area indicating the position of the mesenteron. Size 0·7 × 0·2 mm. When the larva grows the head becomes proportionately smaller and the segmentation more difficult to determine owing to the stretching of the intersegmental membrane. The length, prior to moulting, is 1·1 mm. The head is devoid of larval antennae. The mouth is very small and the mandibles small, but well chitinised. The mouth-parts resemble in general shape those of the third instar larva.

Alimentary canal. The fore-gut is very short and consists mainly of a muscular pharynx. The hind-gut could not be seen, but by examination of the cast larval skin it was possible to trace the chitinous lining of the commencement of the posterior portion of the hind-gut; it is very short and does not communicate with the mesenteron. The salivary glands and the Malpighian tubules could not be seen.

The vascular system resembles that of the third instar larva, but only two chambers of the heart and the terminal bulb of the dorsal vessel are visible. The contractions of the heart are occasional and very feeble.

The tracheal system (Text-fig. I, 1 and 3) consists of a pair of lateral longitudinal trunks extending from the first to the twelfth body segment; they are connected posteriorly by a transverse trunk ventral to the posterior end of the mesenteron. Rudimentary stigmatic trunks are present in body segments I to X. Each rudimentary stigmatic branch gives off two fine terminal branches.



Text-fig. I. *Stenomalus micans.* First instar larva. (1) Lateral aspect. (2) Ecdysis. (3) Tracheae of the head and first thoracic segment.

c. tracheal commissure; *c.f.* caudal furca; *e.* rupture from which the second instar larva emerged; *h.* posterior portion of the head; *l.t.* main lateral longitudinal tracheal trunk; *m.* mesenteron; *n.c.* tract of the nerve cord; *p.* pharynx; *pr.* tract of developing proctodaeum; *st.* 1, first rudimentary stigmatic tracheal trunk; *t.l.* tracheal loop; *v.t.* visceral tracheal trunk.

Spiracles absent. Anteriorly the longitudinal trunk on each side of the body extends dorsally to a small commissure and from each commissure a definite tracheal loop occurs which joins a deeper visceral longitudinal trunk (Text-fig. I, 3). This visceral tracheal system consists of two longitudinal trunks which follow the direction of the main lateral trunks; there are side branches, but these are confined to the mesenteron.

To detect the visceral trunks it is essential to float larvae, which have just hatched, on normal saline for about 6 hours.

General remarks. The larva may be found in any part of the haemocoele of the host, but it appears more usually associated with some part of the host's alimentary canal, keeping in position by means of the caudal furca.

The larva is so small that it is liable to float at random in the blood plasma, but the furca enables it to maintain a definite feeding site. In the later instars, with the increase in body size, the liability to float at random ceases because the larger larva becomes lodged against the host's body wall and gut, etc., the furca being very much smaller or obsolete.

The mouth-parts, with the mandibles moving rapidly, were frequently seen applied to various parts of the contents of the body cavity. The contents of the mesenteron of the late first instar included minute particles of deeply staining matter which suggests that the larva does not feed on the host's blood only, but is partly predaceous.

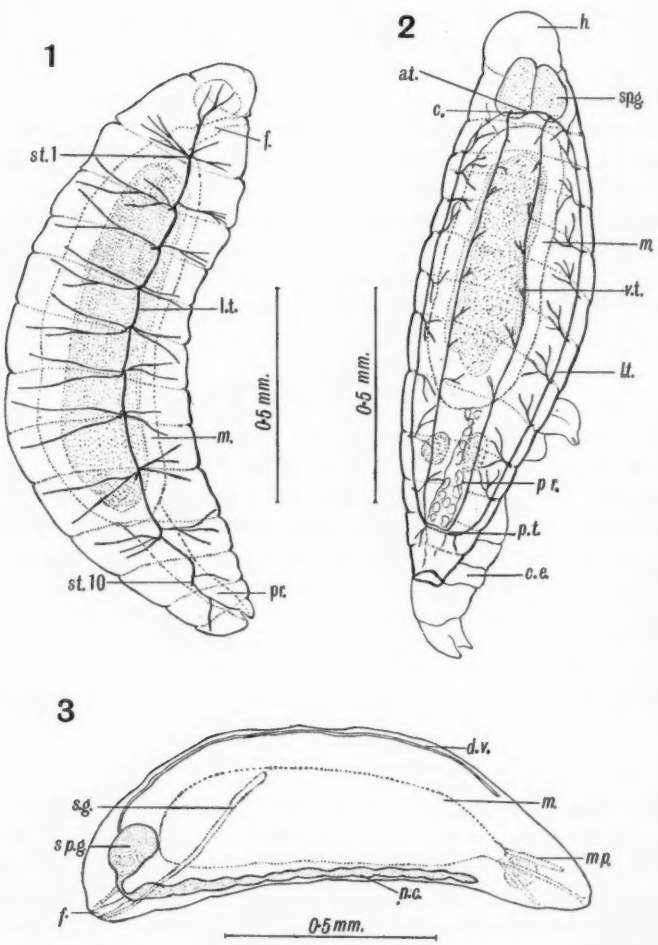
3. MOULTING (Text-figs. I, 2; II, 2).

The process of moulting from the first to second instar larva was followed in larvae placed in normal saline solution and also in specimens left in carefully opened hosts. The time required for the first ecdyses varied, usually 6 hours being needed for freeing all but the last abdominal segment; doubtless, under natural conditions, the process is more rapid. At the start a split occurs in the intersegmental membrane between segments I and II, and is followed by peristaltic waves; the head is quickly freed and, slowly, segment after segment follows. The caudal furca does not seem to assist during moulting. Occasionally, when dissecting a host, moulted skins of the first instar were noticed, and in many cases they had broken down considerably owing to phagocytosis.

4. SECOND INSTAR LARVA (Text-fig. II).

The cuticle is like that of the first instar. The characteristic sickle-shape is lost and the head is now more in proportion to the rest of the body. The second instar larva is 0·9–1·3 mm. long and the late second instar 2·3 mm. long. The caudal furca is absent, but an anal cleft is present. Segmentation is clearly seen in the early second instar, but with the growth of the larva it is more difficult to determine. The larva is not found in any special position of the host's haemocoele, but usually it is maintained in a feeding position by the viscera.

The alimentary canal. The outlines of the fore-gut are more distinct than in the first instar. Two salivary glands are present, one on each side of the oesophagus and occupying an oblique dorso-ventral position; they are parallel to one another and unite to form a short salivary duct which opens into the buccal cavity. The mesenteron occupies the greater part of the haemocoele and a darker central portion is now more noticeable; peristalsis is more regular and definite. The hind-gut is still indefinite, consisting of but a tract of cells. There



Text-fig. II. *Stenomalus micans.* Second instar larva. (1) Lateral aspect. (2) Dorsal aspect of specimen kept on the surface of normal saline solution for 24 hours. (3) Lateral aspect to show the nerve cord.

a.t. anterior transverse tracheal trunk dorsal to the gut; *c.e.* cast exuviae of the first instar larva; *d.v.* dorsal vessel; *f.* tract of the fore-gut; *mp.* Malpighian tubules; *p.t.* posterior transverse tracheal trunk ventral to the gut; *s.g.* salivary glands; *sp.g.* supra-oesophageal ganglia. For other lettering see explanation of Text-fig. I.

is no open communication with the mid-gut. Two Malpighian tubules enter the hind-gut where the mid- and hind-gut subsequently unite.

The vascular system is similar to that of the first instar.

The tracheal system, in general, is like that of the first instar, but the dorsal branches from the lateral longitudinal trunks are much longer, and the rudimentary stigmatic trunks have lengthened, the first pair being shorter than the remainder (Text-fig. II 1, *st.* 1). The dorsal branches are no longer attached to the terminal portion of the rudimentary stigmatic trunks, but to their base or to the main trunk. The tracheal branches of the longitudinal tracheal trunks are associated with each rudimentary stigmatic centre and between each centre there are no tracheal branches. Spiracles are absent.

The nervous system consists of a pair of large supra-oesophageal ganglia and a ventral chain of eleven ganglia.

5. THIRD INSTAR LARVA (Text-fig. III).

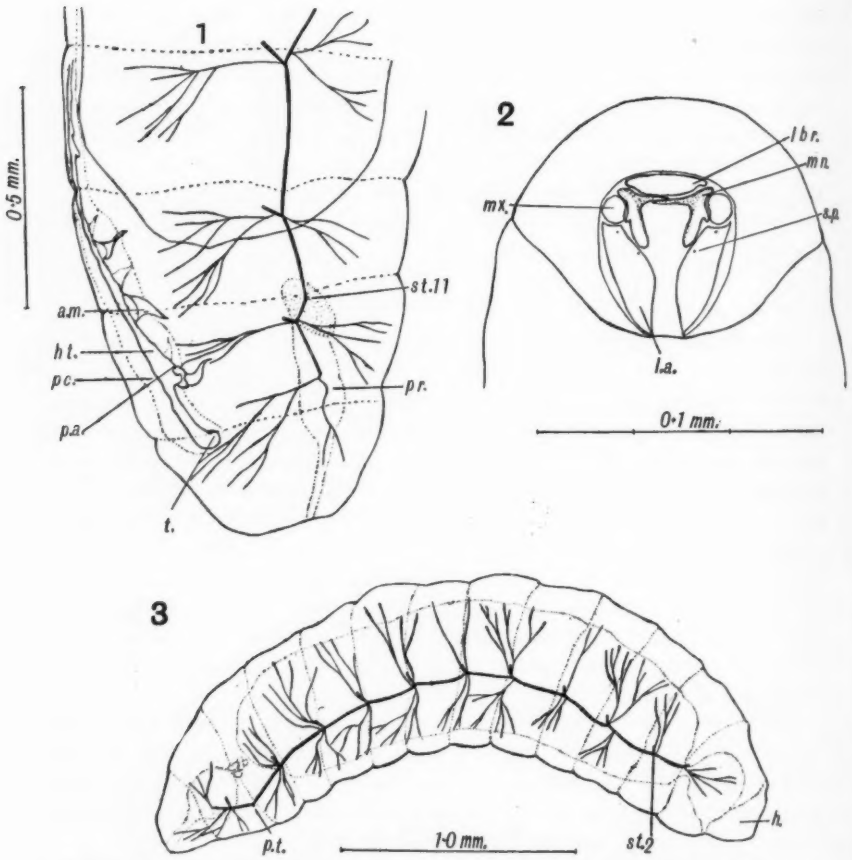
The shape resembles that of the previous instars, but the cuticle appears rough, though without setae. Size 1·6–2 mm. Moulting takes longer, the exuviae often remaining attached to the hind end of the larva, finally becoming loosened with the growth of the larva and consequent mechanical removal. At the end of this instar it is nearly impossible to determine the segmentation of the cuticle, owing to the stretching of the intersegmental membrane. Movement in the host is facilitated by the cuticle being forced into lateral protrusions which occur in the second to seventh body segments. The head (Text-fig. III, 2) appears to be devoid of antennal papillae; the mouth-parts are not well developed, except the mandibles which are very efficient for cutting up minute portions of the host; they are moving continually and are accompanied by a slower but regular sucking action of the pharynx. The labial area bears on each side a pair of (? sensory) pits.

Alimentary canal. The contents of the mesenteron are much darker than in the previous instar. Peristalsis is much slower but regular. The hind-gut shows evidence of development, but there is no communication with the mesenteron. The anal portion of the hind-gut is more developed than the part which will eventually unite with the mesenteron.

The vascular system (Text-fig. III, 1) consists of the dorsal vessel in a pericardium and ends posteriorly in a closed terminal bulb, it then proceeds anteriorly and ends in the dorsal region of the supra-oesophageal ganglia. The vessel is enlarged in body segments Nos. IX–XII to form three chambers, it then narrows into a fine tube and enlarges again in the second and third segments to form two small chambers which are much smaller than the posterior ones. Each chamber of the heart has a lateral alary muscle which is attached to the cuticle of the body wall, whilst there also appears to be an attachment of the chamber to the floor of the pericardium.

Tracheal system (Text-fig. III, 3). Spiracles are absent. The tracheal system is like that of the previous instar, but a very short rudimentary stig-

matic trunk can be seen at the junction of the longitudinal tracheal trunk with the anterior transverse trunk. This makes the total number of rudimentary stigmatic trunks eleven pairs; thus all the body segments, except the last,



Text-fig. III. *Stenomalus micans.* Third instar larva. (1) Posterior segments, lateral aspect. (2) Head and mouth-parts. (3) Lateral aspect.

a.m. alary muscle of a chamber of the "heart"; *ht.* a chamber of the "heart"; *lbr.* labral area; *l.a.* labial area; *mn.* mandible; *mx.* maxillary area; *pa.* pericardial attachment; *pc.* pericardium; *sp.* (? sensory) pits; *st.* 2, second rudimentary stigmatic tracheal trunk, described in the earlier instar as the first; *st.* 11, eleventh rudimentary stigmatic tracheal trunk; *t.* terminal bulb of the dorsal vessel. For other lettering see explanation of the previous Text-figs.

possess a pair. The looping of the longitudinal tracheal trunk at the anterior end (Text-fig. I 3, *t.l.*) in the first instar is lost with the increase in size and age of the larva by the outstretching of the loop, and the commissure has now become the first rudimentary stigmatic trunk. This development necessitated the

re-numbering of the rudimentary stigmatic tracheal trunks; thus in the Text-figures subsequent to and including Text-fig. II the trunk identified as the second (*st.* 2) corresponds to the first (*st.* 1) one in the first instar larva. The average length of the first rudimentary stigmatic trunk is 0·02 mm., that of the second 0·09 mm. A general increase in size and length of the tracheal branches of the main tracheal trunks has taken place. It is impossible, even in a specially starved larva, to see the inner visceral trunk.

6. FOURTH INSTAR LARVA (Text-fig. IV).

At this stage the cuticle of the body wall is thicker and is raised into distendable segmental protrusions which aid the larva considerably to obtain a hold for feeding and body movements, the latter consisting of a spiral twisting.

As soon as the fourth instar is attained the parasite becomes destructive, obviously predaceous and the host soon dies, its cuticle quickly collapsing as the parasite consumes everything except the cuticle, chitinised portions of the mouth-parts and the tracheal system. With this change of feeding habits of the parasite morphological changes occur as follows:

(1) The head (Text-fig. IV, 2) is generally more heavily chitinised, as compared with the rest of the body, in order to support the following structures described under (3) and (4).

(2) The antennae are in the form of small papillae, and consist of a basal ring with a central chitinised spine.

(3) The area between the antennae carries a heavily chitinised boring armature, which is used to break up the viscera and force an exit through the host's cuticle.

(4) The mandibles are enlarged and enclosed within a buccal funnel; they move continually and rapidly.

(5) Development of spiracles.

(6) Size 2·5–3 mm.

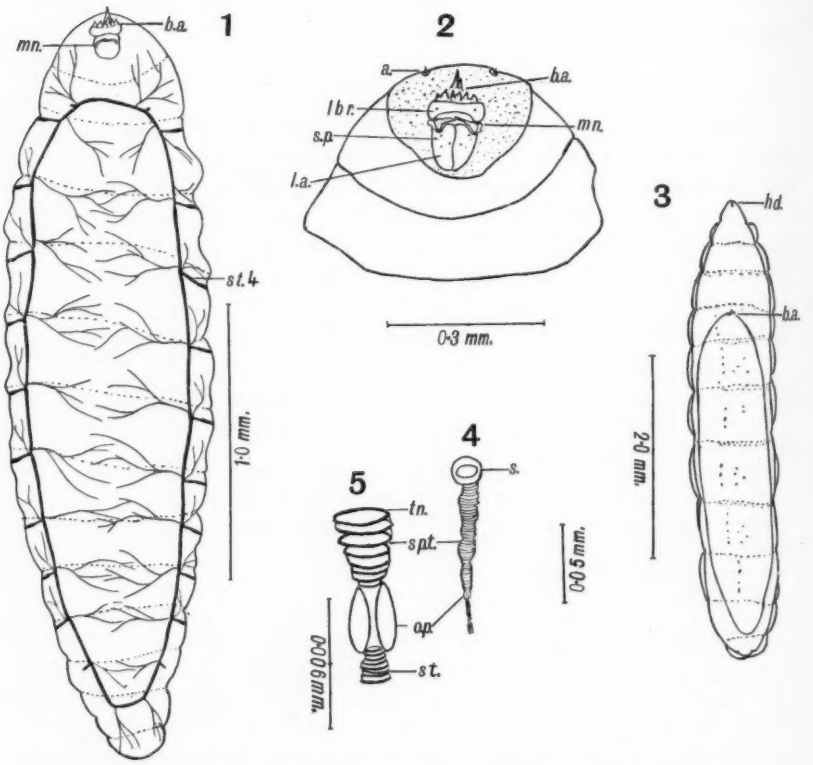
Alimentary canal. No obvious change can be seen by dissection beyond that there is a definite darkening of the mesenteron, the contents of which are often dark green, more especially at the end of the instar. The hind-gut is closed, but development has taken place, a proctodaeal tract of cells being present. In a number of larvae a delicate protrusible vesicle appears from the anus. There are two Malpighian tubules, resting obliquely in segments X–XIII and attached to the gut where the mid- and hind-guts subsequently unite.

Tracheal system (Text-fig. IV, 3). The spiracles have developed and are connected to the former rudimentary stigmatic trunks by short spiracular trunks, details of which are as follows:

Segment I: rudimentary stigmatic trunks still very short, spiracles are absent. Segments II–X: spiracles connected to former rudimentary stigmatic trunks. Segment IV has the longest stigmatic trunks. Segment XI has a short rudimentary stigmatic tracheal trunk, spiracles absent. Segment XII has a transverse tracheal trunk, ventral to alimentary canal, joining longitudinal tracheal trunks.

The branches of the tracheal system are numerous, but in all cases arise near the base of the former rudimentary stigmatic trunks. The spiracles are of

a simple type and consist of a stout peritreme and an oval aperture of 0·02 mm. diameter. The trachea attached to the spiracle is 0·012 mm. in diameter for most of its length, but narrows near its union to the former rudimentary stigmatic trunk to which it is attached by two chitinised oval plates. The diameter



Text-fig IV. *Stenomalus micans*. Fourth instar larva. (1) Ventral aspect. (2) A slide preparation of the ventral aspect of the head. (3) Parasite larva within recently formed puparium of host. (4) Spiracle and stigmatic tracheal trunk. (5) Attachment of spiracular trachea to former rudimentary stigmatic trunk.

a. antennal papilla; *b.a.* boring armature; *hd.* head of host; *o.p.* oval plates; *s.* spiracle; *sp.* (? sensory) pits; *sp.t.* spiracular tracheal trunk; *st.* former rudimentary stigmatic trunk. For other lettering see explanation of previous Text-figs.

of the stigmatic trunk below the plates is 0·002 mm. The necessity for an open tracheal system may be correlated with death of the host and consequent reduction of the oxygen supply. In previous instars no doubt the parasite obtained oxygen which was dissolved in the host's blood plasma.

7. FIFTH INSTAR LARVA.

The larva in this instar is 3·25–3·75 mm. long and resembles externally that of the fourth, but the cuticle is coarser and the intersegmental clefts are deeper; it is opaque.

The longitudinal tracheal trunk is difficult to determine. The dorsal vessel is more distinct and appears as a dark line along the mid-dorsal surface of the body.

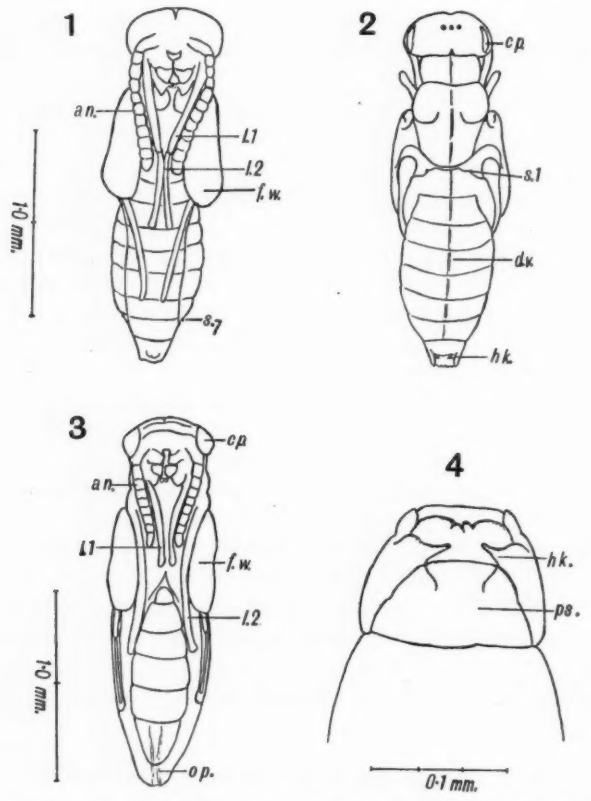
The host (Text-fig. IV, 3) very rarely pupates; usually it reaches the stage at which the puparium is just formed, but it remains colourless (a normal *Chlorops* puparium is reddish brown) and in some cases the larva fails even to commence pupation. It is in nearly all cases immobilised at the lower part of the barley stem just above a node. Normally a gout-fly larva feeds with the head pointing to the root and, prior to pupation, it turns round and moves up the sheath to a point just below where the leaf leaves the stem. This enables the imago to make an easy exit on emergence. A parasitised host larva rarely turns round; it commences to pupate and then the parasite attains its maximum activity and quickly consumes every soft particle of the host's body. The parasite, still in the fourth instar, bores an exit hole through the pupal-larva cuticle which splits at the intersegmental membrane between segments II–III and the parasite wriggles out. It is interesting that the head of the parasite and that of the host are pointing in the same direction. The parasite now moves a short distance from the host's remains and turns round, so that the head is in the direction of the growing point of the barley. It spins from left to right across the interior of the stem a few strands of silk, secreted by the salivary glands. It rests for a few hours and then moults (including the lining of the newly developed proctodaeum). A period of defaecation follows and at first a few drops of transparent fluid are exuded from the anus followed by a few pear-shaped faeces, and about 12–18 hours later the remaining contents of the mesenteron are voided.

8. PUPA (Text-fig. V) AND EMERGENCE OF IMAGO.

Following the act of defaecation by the larva the prepupal instar commences. This lasts for 3 days and then the larval cuticle splits and shrivels off the posterior end of the pupa. The pupa is at first pale yellow and gradually darkens towards the completion of the pupal instar. It remains yellowish brown for 6–7 days after which it turns very dark; this darkening starts on the thorax and extends gradually to the abdomen, then within 24 hours after attaining a general dark colour it changes to an intense black. Emergence occurs 4–6 days after this final coloration. The gradual change in colour, more especially the degree of pigmentation of the compound eyes, is a reliable index for the determination of the age of the pupa, being correct to within a day. The rate of pigmentation was the same for pupae exposed to light and for those retained within the barley stem.

The description of the pupa is as follows:

The early pupa (*up to* 7 *days old*) has the usual external features of a Chalcid pupa. The spiracles on the meso- and meta-thorax are small and difficult to see. The spiracles of the first apparent abdominal segment are prominent, and those



Text-fig. V. *Stenomalus micans*. Pupae. (1) Ventral aspect. (2) Dorsal aspect of ♂ pupa, five days after pupation. (3) Ventral aspect of ♀ pupa, seven days after pupation. (4) Dorsal aspect of the terminal portion of the abdomen of ♂ pupa.

an. antenna; *cp.* developing compound eye; *f.w.* fore-wings; *hk.* abdominal hooks; *l.* 1, first thoracic leg; *l.* 2, second thoracic leg; *op.* developing ovipositor; *ps.* posterior segment; *s.* 1, first apparent abdominal spiracle; *s.* 7, seventh apparent abdominal spiracle. For other lettering see previous Text-figs.

on the seventh apparent abdominal segment are mounted on short pedicels and are nearly as large as those of the first abdominal segment. On the last segment a pair of hook-like structures (Text-fig. I, 2 and 4) can be seen from the dorsal surface of the male, but are less distinct in the female because they are more terminal in position.

Sex characters of the pupa. The male. Size 3·4–3·6 mm. × 1 mm. The antennal sheath is longer than that of the female and extends as far as seven-eighths of the length of the wing pad; there are eleven apparent joints. The first thoracic legs extend as far as the tenth apparent antennal joint, the second leg pair as far as abdominal segment III and the third leg pair as far as abdominal segment VI.

The female. Size 3·5–4·0 mm. × 1·1 mm. The antennal sheath extends as far as three-eighths of the length of the wing pads and there are eight apparent joints. The first pair of legs extends beyond the terminal joint of the antennae. The genitalia are insufficiently developed to determine the sex by this character alone; in some pupae the developing ovipositor appears as two closely apposed lines which are slightly darker than the rest of the abdomen.

Late pupa and emergence of the imago. The developing imago can be seen within the pupal sheath and there is a general loosening of the body and appendages from the pupal cuticle. The sexes can be distinguished easily, as the ovipositor, which occupies the greater length of the ventral surface of the abdomen, is very conspicuous. Immediately prior to emergence vigorous abdominal movements occur and the two hook-like structures appear to provide the imago with a means of fixing the pupal sheath and assist the efforts of bursting the cuticle, which splits dorsally and is then slipped off. In specimens bred in tubes, the pupal skin tends to remain on the posterior segments of the abdomen, but under natural conditions the skin is fixed to the inside of the barley stem. The adult Chalcid is enclosed in the tunnel made by the feeding of the *Chlorops* larva in the barley stem and within this it rests until the cuticle is completely hardened and the wings fully developed. An exit hole is then cut with a pair of well-developed mandibles and usually the Chalcid has to cut through 4–5 layers of ensheathing leaves; the holes, more especially in wet weather, are difficult to find in the field, as they tend to close up owing to the continued growth of the galled stem. Under laboratory conditions the holes are easily found in dried stems of "gouted" barley. Emergence occurs 14 days after pupation and the males and females emerge at the same date (most of the emergences in 1928 occurred between the first and third weeks in August). After slight defaecation flight takes place and the imagines feed on the exudations on the stems of green tillers of barley. They have been also captured in numbers from various Umbelliferae in the hedgerows around the field. Mating is a very short process, after which the females seek young *Chlorops* larvae in which they lay their eggs.

9. SUMMARY.

1. Two species of endoparasites, a Chalcid *Stenomalus micans* and a Braconid *Coelineus niger*, were found in every sample of "gouted" barley examined from a number of counties in southern England.

2. *Chlorops* infestations were severe in 1928 in many districts, and the majority of the "gouted" shoots were of winter-type damage, of which 68 per cent. were parasitised, two-thirds by *S. micans*.

3. The larval anatomy of *S. micans* is described:

(*a*) There are five larval instars, each of which is described.

(*b*) The first larval instar appears to be partly predaceous.

(*c*) The larvae can be kept alive for 5 days on the surface of normal physiological saline solution and moulting occurs, which enables the instar to be determined with certainty.

(*d*) The tracheal system is devoid of spiracles until the fourth larval instar; spiracles then develop and are connected to the former rudimentary stigmatic trunks prior to the death of the host.

(*e*) The fourth instar larva develops a cephalic boring armature which is used for breaking up the internal organs of the host and also to bore an exit hole through the latter's body wall.

4. The pupa of *S. micans* is described and sex differences are indicated.

Acknowledgments.

A brief outline of the life histories of *Stenomalus micans* and of the other parasites of the gout-fly is given in a paper, to be published shortly, by Dr J. G. H. Frew, to whom I am much indebted for suggesting that I should investigate the problem to which this paper relates, after our preliminary study thereof was carried out at Rothamsted Research Station in 1926. My sincere thanks are due to Dr A. D. Imms, F.R.S., for providing me with laboratory facilities and for granting me permission to collect material from the Experimental Plots (Hoos Field).

The late Dr J. Waterston identified the species of Chalcids, and the late Mr G. Lyle the Braconids. Both of these gentlemen laboured at overcoming certain difficulties of identification and I take this opportunity of expressing my appreciation for the help they gave me. Finally, my thanks are due to Messrs A. C. Evans and F. W. Wakefield for ready help they have given me, especially in the tedious work of collecting and examining material.

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SOME DIGENETIC TREMATODES OF MARINE FISHES OF BEAUFORT, NORTH CAROLINA[1].

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(With 25 Figures in the Text.)

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[1] Studies from the Zoological Laboratory, the University of Nebraska, No. 169.

IN 1928, a collection of marine fish trematodes was made at the United States Bureau of Fisheries Station at Beaufort, North Carolina; thirty-six of fifty-one species of fishes examined contained trematodes; only one or two specimens of some species were examined. Only Monogenea were collected from Elasmobranchs. The following report includes the digenetic forms identified. Type specimens of all new species are deposited in the United States National Museum.

Order **GASTEROSTOMATA** Odhner.

1. **Nannoenterum baculum** (Linton 1905) n.comb.

Synonym: *Gasterostomum baculum* Linton 1905.
Host: *Scomberomorus maculatus*, Spanish mackerel.
Position: intestine. Frequency: common.

2. **Prosorhynchus crucibulum** (Rud.) Odhner 1905.

Host: *Pomatomus saltatrix*, bluefish.
Position: gill region. Frequency: one from eleven hosts.

3. **Bucephalopsis gracilescens** (Rud.).

Host: *Menidia menidia*, silversides.
Position: intestine. Frequency: not common.

Order **PROSOSTOMATA** Odhner.

Family ASPIDOGASTRIDAE Poche.

4. **Aspidogaster ringens** Linton 1905.

Host: *Micropogon undulatus*, croaker.
Position: intestine. Frequency: common.

5. **Macraspis** sp., immature (probably *M. elegans*).

Host: *Menticirrhus americanus*, whiting.
Position: intestine. Frequency: three in one of four hosts.

Family FELLODISTOMIDAE Nicoll.

Sub-family FELLODISTOMINAE.

6. **Steringotrema corpulentum** (Linton 1905) n.comb. (Fig. 1).

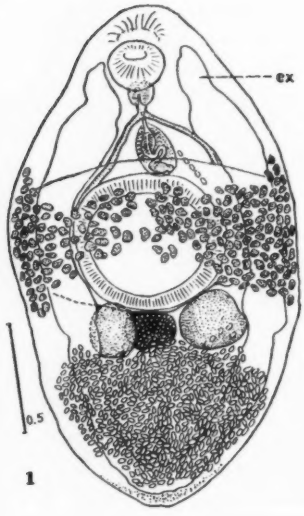
Synonym: *Distomum corpulentum* Linton 1905.
Host: *Lagodon rhomboides*, pinfish.
Position: intestine. Frequency: common.

Linton's (1905, p. 382) description is correct except in regard to diverticula on the intestinal caeca. These do not occur. In living specimens branches of the excretory system might easily be confused with branches from the caeca. The thick, plump body, the distribution of the vitellaria and the median genital pore distinguish the species from others in the genus. If the genus *Pycnadena* Linton 1910 should be retained as distinct from *Steringotrema*, this species should probably be considered a second species of *Pycnadena* because of its median genital pore.

Family MONORCHIDAE Odhner.

Genus **Genolopa**.

Odhner (1911, p. 252) considered Linton's *Genolopa ampullacea* and *Genolopa truncata* as belonging to different sub-families. A study of Linton's two species



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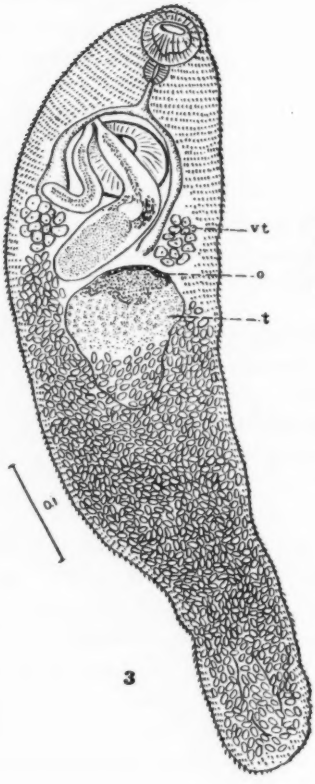

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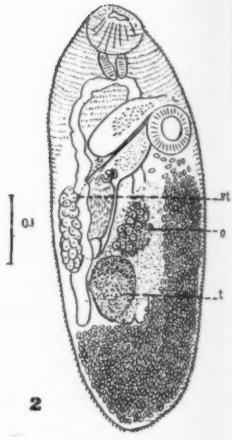

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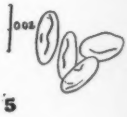

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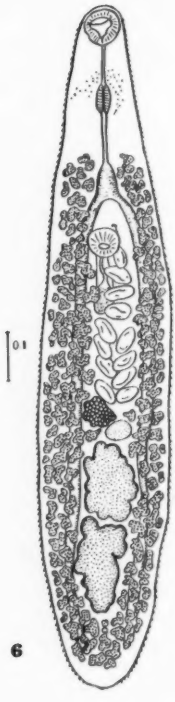

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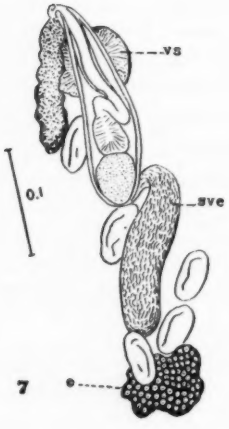

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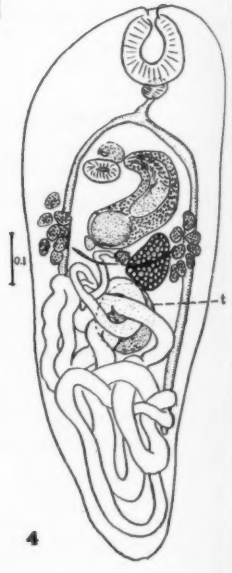

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shows they are actually very similar except in caeca length, and they are here considered as both in the genus *Genolopa*. Two new species are herein described, these, with Nicoll's (1915) *G. trifolifer* and *G. cacuminata*, make a present total of six species in the genus. In every case, a given fish host supports two species of *Genolopa*, usually simultaneously.

7. **Genolopa minuta** n.sp. (Fig. 2).

Synonym: *Monostomum* sp. Linton 1905, p. 356.
Hosts: *Menidia menidia*, silversides; *Fundulus majalis*, minnow.
Position: intestine. Frequency: common in silversides.

Body oval or slightly elongate, very small (0·35–0·63 mm. × 0·17–0·63 mm.) covered with spines. Oral sucker slightly larger than ventral sucker (*e.g.* 59 and 51μ; 56 and 48μ). Genital pore slightly to the left close in front of ventral sucker. Pre-pharynx lacking; pharynx almost spherical, oesophagus lacking or very short; caeca (weakly developed and usually covered by eggs) terminate a short but appreciable distance in front of the posterior end. Testis single, median, oval or elongate, slightly posterior to mid-body. Cirrus sac large, clavate, extending posterior to the ventral sucker. Cirrus armed with spines. Ovary oval, often inconspicuous, anterior to testis. Vitelline glands inconspicuous, forming two compact groups of cells, dorsal, just posterior to ventral sucker. Eggs oval (16–17 × 7–8μ) filling almost the entire body and covering nearly all the reproductive organs. Metraterm conspicuous, clavate, spined, considerably shorter than cirrus sac. Excretory system with short median stem.

One live specimen only 0·19 mm. in length was filled with eggs. No other internal organs could be made out.

This species differs from *Genolopa ampullacea*, to which it is most similar, in its more elongate body, relative sizes of the suckers, more posterior extent of the cirrus sac, and shorter caeca.

8. **Genolopa elongata** n.sp. (Fig. 3).

Synonym: *Distomum* sp. Linton 1905, p. 360.
Host: *Menidia menidia*, silversides.
Position: intestine. Frequency: common.

Explanation of Text-figures 1–25.

All figures were drawn with the aid of a camera lucida. The value in millimetres of the projected scale is indicated in each figure.

Lettering: *ex*, excretory system; *ga*, genital atrium; *gp*, genital pore; *ic*, intestinal cecum; *o*, ovary; *ovg*, opening of ventral groove; *sr*, seminal receptacle; *sv*, seminal vesicle; *sve*, external seminal vesicle; *svi*, internal seminal vesicle; *t*, testis; *vg*, ventral groove; *vs*, ventral sucker; *vt*, vitellaria.

Figures 1–7.

Fig. 1. *Steringotrema corpulentum* from *Lagodon rhomboides*, ventral view.
Fig. 2. *Genolopa minuta* from *Menidia menidia*, ventral view.
Fig. 3. *Genolopa elongata* from *Menidia menidia*, dorsal view.
Fig. 4. *Proctotrema lintoni* from *Menidia menidia*, ventral view.
Fig. 5. *Proctotrema lintoni*, eggs.
Fig. 6. *Pharyngora gracilis* from *Menidia menidia*, ventral view.
Fig. 7. *Pharyngora gracilis*, dorsal view of the terminal reproductive organs.

Body elongate (*e.g.* 0·924 × 0·168 mm.), spined. Ventral sucker always larger than oral sucker (*e.g.* 57 and 42μ). Genital pore slightly to the left just in front of ventral sucker. Pre-pharynx lacking, oesophagus about as long as the pharynx. The caeca seem to be very short. They could not be traced very far posterior to the ventral sucker and the dense mass of eggs filling the entire posterior half of the body gives no indication of other organs. The large testis overlaps the ovary in front of it. Both cirrus and metraterm are spined. The metraterm is more elongate than in other species. The two inconspicuous groups of vitelline cells are lateral and just posterior to the ventral sucker. Eggs measure 16–19 × 7–9μ. The median stem of the excretory system is longer than in *G. minuta*.

This species is very distinct. It resembles *G. cacuminata* in body shape and sucker ratio. This latter species, however, possesses a distinct pre-pharynx, median genital pore, vitellaria in two lateral rows, and larger eggs.

9. **Proctotrema lintoni** n.sp. (Figs. 4 and 5).

Synonym: *Monostomum* sp. Linton 1905, p. 379.

Hosts: *Orthopristis chrysopterus*, hogfish; *Menidia menidia*, silversides.

Position: intestine. Frequency: one from nineteen hogfish; one from eighteen silversides.

These two specimens were finally referred to the same species although there are certain slight differences. Both specimens seemed to be somewhat macerated and only a few spines could be seen. Spines are probably normally present. Fig. 4 is of the specimen from silversides. The other specimen (from hogfish) is similar except for an oral sucker even more drawn out posteriorly, and a three-lobed ovary.

Body elongate, tapering almost to a point posteriorly, broadest anteriorly, 1·1 mm. in length and 0·45 mm. greatest width. Oral sucker elongated posteriorly, about twice the size of the ventral sucker (diameters 144 and 86μ respectively). Genital pore median, just anterior to the ventral sucker. Pharynx small, almost spherical; oesophagus short; caeca not reaching the posterior end by a short distance. Testis single, median, spherical. Cirrus sac large, extending some distance posterior to ventral sucker; cirrus spined, prostate cells conspicuous, spherical seminal vesicle in base of cirrus sac. Ovary anterior and to the right of testis. Vitelline glands in two lateral groups of about eight follicles each, posterior to ventral sucker. A small seminal receptacle seems to be present. Uterus fills posterior part of body. Eggs about 24 × 10μ (according to Linton three times longer than wide).

The shape of the eggs readily distinguishes this species from *Proctotrema bacilliovatum* Odhner 1911.

Family ACANTHOCOLPIDAE Odhner.

10. **Stephanochasmus casus** Linton 1910.

Host: *Micropogon undulatus*, croaker.

Position: intestine. Frequency: three in two of thirty-one hosts.

11. **Stephanochasmus dentatum** (Linton).
Host: *Paralichthys dentatus*, summer flounder.
Position: intestine. Frequency: common.

Family ALLOCREADIIDAE Stossich.

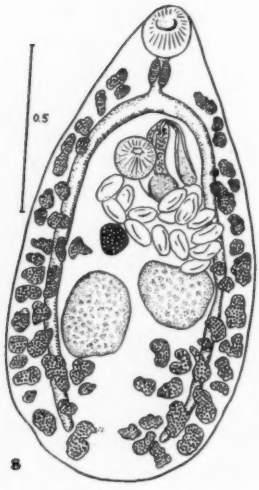
Sub-family LEPOCREADIINAE.

12. **Lepocreadium ovalis** n.sp. (Fig. 8).
Synonym: *Distomum* sp. Linton 1901, p. 458.
Host: *Lagodon rhomboides*, pinfish.
Position: intestine. Frequency: not common.

This species is named tentatively being represented by only one specimen and several smaller, similar forms, which may represent a different species. The body is flattened, oval, tapering anteriorly, broadly rounded posteriorly, widest near the posterior end. It measures 1·3 × 0·75 mm. Spines are apparently absent, but might have been originally present. The oral sucker is larger than the ventral sucker (0·144 and 0·128 mm.) which is located about one-third from the anterior end. The genital pore is well anterior to the ventral sucker, slightly to the left, at about the level of the forking of the caeca. A very short pre-pharynx, and short oesophagus are present. The caeca fork about half-way between the suckers and extend to near the posterior end of the body. Testes large, oblique, just posterior to mid-body; cirrus sac well developed, clavate, extending a short distance posterior to the ventral sucker; external and internal seminal vesicles present. Ovary unlobed, just anterior to the right testis; seminal receptacle present; uterus short, entirely anterior to the testes. Eggs relatively few and large (96 × 56μ). Metraterm well developed, to the left of cirrus sac, extending posteriorly to about the level of the posterior border of the ventral sucker. The vitelline glands consist of large follicles extending forward to the pharynx and backward to the posterior end of the body. This trematode resembles the genus *Lebouria* in body form and in extent of vitellaria, but differs in posterior extent of the cirrus sac and in small size of ventral sucker. It differs from most other *Lepocreadium* species in anterior extent of vitellaria and in body form and egg size.

13. **Pharyngora gracilis** n.sp. (Figs. 6 and 7).
Host: *Menidia menidia*, silversides.
Position: intestine. Frequency: common.

Body spined, elongate, very thin, gradually tapering anteriorly, more broadly rounded posteriorly (0·84–1·5 × 0·18–0·4 mm.). Suckers almost exactly equal in diameter, ventral sucker sometimes very slightly larger. Genital pore at left anterior edge of ventral sucker. The oral sucker is slightly elongated posteriorly, but this peculiarity is not noticeable in all specimens. Pigment granules on each side dorsally just anterior to pharynx. Pre-pharynx, oesophagus, and pseudo-oesophagus present. In a 1·01 mm., extended specimen, the length of the pre-pharynx was 72μ, pharynx 68μ, oesophagus 83μ, pseudo-oesophagus 48μ. Testes distinctly or slightly lobed, tandem, in posterior half



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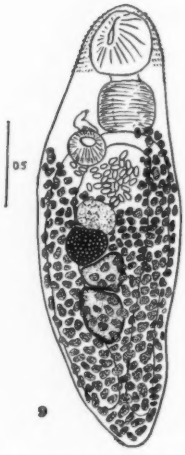

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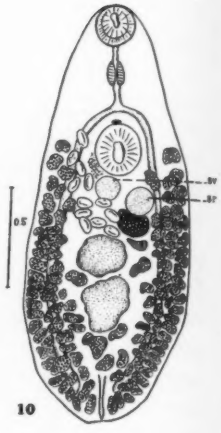

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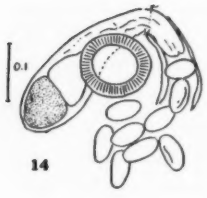

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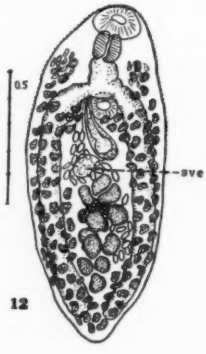

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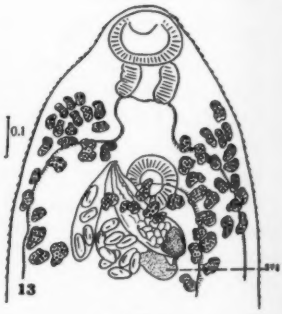

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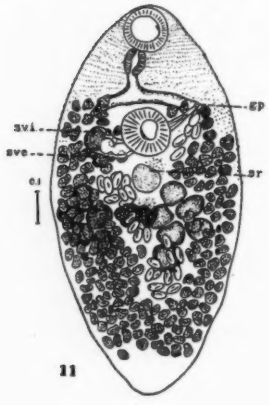

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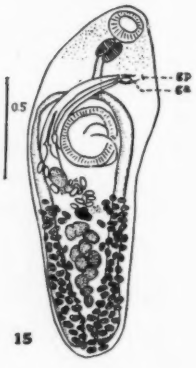

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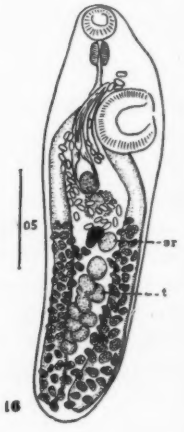

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of body. Cirrus sac elongate, extending posterior to the ventral sucker either a short distance or sometimes half-way to ovary. Spherical internal seminal vesicle and elongate external seminal vesicle present (Fig. 7). Cirrus unspined. Ovary median, slightly lobed, a little posterior to mid-body; seminal receptacle posterior to ovary; uterus anterior to ovary; eggs large, 64–72 × 39μ (even in the 0·84 mm. specimen, eggs were 64 × 39μ). Vitellaria well developed, lateral, confluent posteriorly, extending forward to anterior limit of pseudo-oesophagus. This anterior extent of the vitellaria is the chief difference between this species and *Pharyngora bacillaris* in which the vitellaria do not even reach the ventral sucker.

14. **Lepidauchen hysterospina** n.sp. (Fig. 9).

Synonym: *Distomum* sp. Linton 1905, p. 382.
Host: *Lagodon rhomboides*, pinfish.
Position: intestine. Frequency: three in one of twenty-seven hosts.

Body elongate (2·54 × 0·87 mm.); spined only in region of oral sucker and at the extreme posterior tip. Ventral sucker one-third from anterior end, only about half the diameter of the oral sucker (0·24 mm. as compared with 0·42 mm.). Genital pore a short distance anterior to ventral sucker about at mid-pharynx level, slightly to the left. Short pre-pharynx; very large pharynx (0·38 × 0·35 mm., or much larger than ventral sucker); oesophagus lacking; broad caeca reaching to near the posterior end. Testes tandem, in posterior half of body; cirrus sac elongate, extending only shortly beyond the anterior border of the ventral sucker. Sections seem to show that the metraterm enters the cirrus sac to form a short genital sinus as Nicoll inferred for *Lepidauchen stenostoma*. Seminal vesicle entirely within the cirrus sac. Ovary unlobed, about in mid-body. What appears as a seminal receptacle is a swollen region of the uterus. Laurer's canal present. Uterus entirely anterior to the ovary. Eggs fairly large (72–75 × 36–38μ), not numerous. Vitelline follicles large, numerous, overlapping the gonads and filling most of the body posterior to the pharynx. Median stem of the excretory system branches at about the level of the middle of the ovary.

This species differs from *L. stenostoma* in distribution of spines, in more elongate cirrus sac, in distribution of vitelline follicles, and in its somewhat smaller egg.

Figures 8–16. For lettering, see p. 399.

Fig. 8. *Lepocreadium ovalis* from *Lagodon rhomboides*, ventral view.
Fig. 9. *Lepidauchen hysterospina* from *Lagodon rhomboides*, ventral view.
Fig. 10. *Homalometron pallidum* from *Fundulus majalis*, ventral view.
Fig. 11. *Multitestis inconstans* from *Chaetodipterus faber*, ventral view.
Fig. 12. *Multitestis blennii* from *Hypsoblennius hentz*, dorsal view.
Fig. 13. *Multitestis blennii*, dorsal view of anterior portion.
Fig. 14. *M. blennii*, ventral view of the region of the genital pore showing cirrus inserted in metraterm.
Figs. 15, 16. *Rhagorchis odhneri* from *Ceratacanthus schoepfii*, ventral view.

15. **Homalometron pallidum** Stafford (Fig. 10).

Synonyms: *Distomum* sp. Linton 1901, p. 442; *Distomum globiporum* Rud. of Linton 1905, e.p. (*Lepocreadium*) *serospinosum* sp.inq. Nicoll 1909.

Hosts: *Fundulus heteroclitus*, minnow; *Fundulus majalis*, minnow; *Leiostomus xanthurus*, spot.

Position: intestine. Frequency: rather common.

This species differs from *Lepocreadium* in the absence of a cirrus sac. The form from *Leiostomus xanthurus* was finally decided to be the same species. Although more elongate, it has the same general characteristics, similar sucker ratio, position of genital pore, extent of vitellaria, egg size, and also lacks a cirrus sac. *Homalometron pallidum* is not the only *Lepocreadium*-like trematode lacking a cirrus sac. The genus *Microcreadium* Simer 1929 is a related genus not only lacking a cirrus sac but possessing a median genital pore. Simer names a new genus, *Anallocreadium*, for *Allocreadium armatum* (MacCallum 1895) because it lacks a cirrus sac.

Multitestis n.gen.

16. **Multitestis inconstans** (Linton 1905) n.comb. (Fig. 11).

Synonym: *Distomum inconstans* Linton 1905.

Host: *Chaetodipterus faber*, porgee.

Position: intestine. Frequency: five in two of seventeen hosts.

The following description combines the data of Linton (1905, p. 400) with some additions.

Body shape from nearly circular to elongate-oval, flattened (0·7–2·1 × 0·45–0·77 mm.); body spined anteriorly (as far back as the region of the ovary). Suckers about equal in size, one or the other being slightly larger. Very short pre-pharynx; oesophagus short; caeca extending to near the posterior end. Genital pore close in front of ventral sucker, varying from only slightly to the left to a point nearly half-way to the lateral border. Testes numerous, in two lateral groups, usually six on the left, four on the right; sometimes six on the left, five on the right. Cirrus sac conspicuous, clavate, passing diagonally backward and to the right from the genital pore. It may overreach the ventral sucker only slightly or appreciably. Internal and external seminal vesicle present. Ovary median, four- or five-lobed; seminal receptacle anterior to ovary; vitelline follicles abundant, lateral, extending to posterior end of body and anteriorly to forking of the caeca. Linton describes them as sometimes reaching the pharynx. Such was not the case in any of my five specimens. In living material, the follicles might be confused with glands near the anterior end. The vitelline follicles are dorsal to the ovary, uterus, and cirrus sac, but ventral to the testes. The uterus extends posteriorly a short distance beyond the testes, then anteriorly to the genital pore. Eggs few and large (56–64 × 29–34μ; according to Linton: 60 by 40μ).

Diagnosis of genus Multitestis. Allocreadiidae. Allocreadiinae.

Body oval or slightly elongate, spined anteriorly. Ventral sucker smaller or

approximately the same size as oral sucker. Genital pore just anterior and to the left of ventral sucker. Pharynx and oesophagus well developed. Testes numerous, usually ten or eleven, median, or in two lateral groups. Cirrus sac well developed. External and internal seminal vesicles present. Ovary median, four- or five-lobed. Vitellaria follicular, well developed, extending to posterior end of body and at least as far forward as the ventral sucker. Uterus extends posterior to ovary. In intestine of marine fishes. Type species: *Multitestis inconstans* (Linton).

The genus *Multitestis* shows its relationship to the Allocreadiinae chiefly through the genus *Rhagorchis* described below.

17. **Multitestis blennii** n.sp. (Figs. 12–14).

Hosts: *Hypsoblennius hentz*, blenny; *Hypleurochilus geminatus*, blenny.
Position: intestine. Frequency: not common.

Body shape elongate-oval, rather pyriform in young specimens; 0·9–1·29 × 0·4–0·59 mm. Anterior half of body covered with spines. Oral sucker larger than ventral sucker (ratio about 3 : 2). Genital pore close to the left anterior border of the ventral sucker. Pre-pharynx lacking; pharynx large, almost as broad as long; oesophagus about equal to pharynx in length; caeca large, reaching to the posterior end of body. Testes ten to eleven (usually eleven) distributed in a single median group in posterior half of body, dorsal, extending anterior and posterior to ovary. Cirrus sac clavate as in *M. inconstans*; external and internal seminal vesicles present. In two instances, the protruded cirrus was found to be inserted into the metraterm a distance of about 0·1 mm. (Fig. 14). Thus, self-fertilisation probably occurs. Ovary lobed, median, somewhat posterior to mid-body. Seminal receptacle present. Vitelline follicles extending from the level of pharynx to posterior end of body, uniting posteriorly and sometimes behind the ventral sucker (Fig. 13). Posterior extent of uterus variable, sometimes reaching only the posterior border of the ovary, sometimes reaching some distance beyond this point. Eggs 54–64 × 32–40μ.

This species differs from *Multitestis inconstans* in distribution of testes and in relative sizes of suckers. It is very similar to *Distomum* sp. from Chaetodon described from Bermuda by Linton (Linton 1907, p. 115, Fig. 69). This latter species evidently belongs in the same genus.

18. **Rhagorchis odhneri** n.gen., n.sp. (Figs. 15 and 16).

Host: *Ceratacanthus schoepfii*, fool fish.
Position: intestine. Frequency: two in one host.

Body elongate-oval, broadly rounded at both ends, without spines, widest at about the level of ventral sucker, 1·75–2 × 0·56–0·59 mm. Ventral sucker about one-third or a little less from the anterior end, larger than oral sucker (ratio, 1·5–2 : 1). Genital pore near the left body margin about half-way between the suckers. Pre-pharynx lacking, pharynx well developed, oesophagus longer than the pharynx; large caeca reaching to posterior end. Eleven testes,

median, extending as an irregular longitudinal group from a point immediately posterior to the ovary to near the posterior end. A genital atrium is well developed (Fig. 15); cirrus sac conspicuous, elongate-clavate, extending from the genital atrium in a broad curve around the right edge of the ventral sucker to about half-way between the ventral sucker and ovary. Internal and external seminal vesicles present, the latter surrounded by a conspicuous group of cells which probably represents the prostate gland. Ovary median, some distance posterior to ventral sucker, four-lobed. Seminal receptacle latero-posterior to ovary. Uterus entirely anterior. Eggs 59–64 × 35μ, relatively few. Metraterm muscular, extending from genital atrium to near the posterior border of ventral sucker. Vitelline follicles well developed, lateral, mostly ventral to caeca, continuous posteriorly, extending anteriorly only to the ovary, being therefore limited to the posterior half of the body. Excretory bladder a long median tube extending forward to the ventral sucker; excretory pore dorsal, near the posterior end.

Rhagorchis is similar to *Podocotyle* in body form, sucker ratio, long oesophagus, four-lobed ovary, metraterm, cirrus sac, position of uterus, and in its excretory system. It differs in number of testes, external seminal vesicle with prostate cells, and in its long genital atrium. *Rhagorchis* is clearly related to the genus *Multitestis*, but differs in sucker ratio, unspined body, anterior uterus, genital atrium, and also by the ovary being anterior to all the testes.

Diagnosis of genus Rhagorchis. Allocreadiidae. Allocreadiinae.

Numerous testes in single median group. Genital pore well to the left about midway between the suckers. Cirrus sac extending posterior to ventral sucker. External seminal vesicle surrounded by gland cells. Ovary median, lobed, anterior to testes. Uterus anterior to ovary. Eggs without filament. Well developed genital atrium. Type species: *Rhagorchis odhneri* from intestine of *Ceratocanthus schoepfii* at Beaufort, N.C.

Family HEMIURIDAE Lühe.

Sub-family HEMIURINAE.

19. **Hemiurus appendiculatus** (Rud.).

Host: *Opisthonema oglinum*, hairy back.
Position: stomach. Frequency: one from three hosts.

Sub-family STERRHURINAE.

20. **Sterrhurus monticellii** (Linton) (Fig. 17).

Hosts: *Pomatomus saltatrix*, bluefish; *Paralichthys albiguttus*, summer flounder; *Centropristes striatus*, black sea bass.
Position: stomach. Frequency: rather common.

The ventral sucker is from about three to a little over four times larger than the oral sucker. Four pairs of oral papillae and a pair of papillae lateral to the oral sucker were seen on a living specimen from the bluefish.

21. **Lecithochirium synodi** n.sp. (Figs. 18–20).

Hosts: *Synodus foetens*, lizard fish; *Paralichthys dentatus*, summer flounder.
Position: stomach. Frequency: many in lizard fish, few (immature) in flounder.

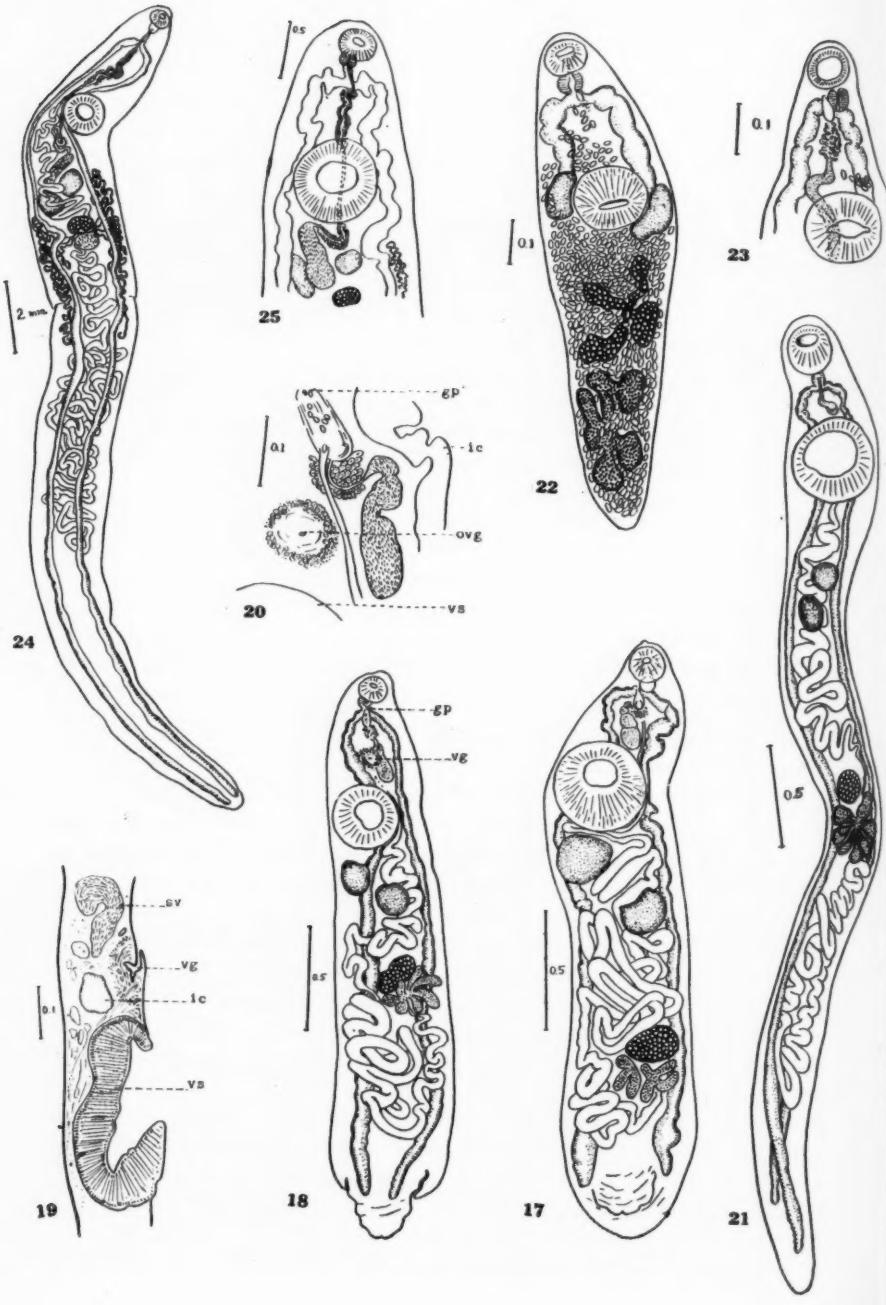
Body size, with tail appendage either wholly or partly retracted, 1·5–4·8 × 0·5–0·8 mm. (a 2·38 mm. specimen possessing a tail appendage of 0·5 mm.); unspined; without rings; widest near the base of the tail. Ventral sucker at about the end of first body fourth, very slightly more than twice the size of oral sucker. Oral sucker without internal elevations. The ventral groove or pit is median, a short distance anterior to the ventral sucker. Numerous cells around it give it a glandular appearance. Genital pore at level of posterior border of pharynx. Testes, ovary, uterus, and vitellaria are characteristic for the genus. The seminal vesicle is divided into three parts and seems to lead directly into the **S**-shaped pars prostatica. The seminal vesicle reaches posteriorly to about the anterior border of the ventral sucker or a little beyond. Sinus sac claviform, broadening gradually to its base. The uterus never enters the tail appendages, and the caeca only occasionally do so for a short distance. Eggs 12–16 × 7–9μ.

This species differs from *L. rufoviride* and *L. gravidum* in shape of oral sucker and direct pars prostatica. It also differs from *L. rufoviride* in relative sizes of suckers. It is perhaps most similar to *L. gravidum*, but differs in lacking the elevations in the oral sucker, in having a definite sucker ratio, and in having a considerably smaller egg. Linton collected similar forms from the same hosts and referred them to *Dist. monticellii*. They apparently lacked the ventral cervical pit.

22. **Hysterolecitha elongata** n.sp. (Fig. 21).

Host: *Mugil cephalus*, mullet.
Position: stomach. Frequency: two in one of six hosts.

Body elongate, cylindrical, orange-red in colour, without tail appendage, unspined, without rings, widest at level of ventral sucker (which is relatively far forward), tapering toward both ends, becoming narrowly pointed posteriorly, size 4–4·7 × 0·49 mm. Ventral sucker twice the size of oral sucker. Oral sucker with three papillae on each side of the mouth, middle pair farther lateral than the others. Genital pore median, close behind the oral sucker. Pre-pharynx and oesophagus lacking; caeca reaching to near the posterior end of body. Testes close together diagonally, not far behind the ventral sucker, not separated by the more dorsal uterine coils. The tubular seminal vesicle extends only to the anterior border of the ventral sucker and is divided into three parts rather indistinctly; pars prostatica short; sinus sac cylindrical, weakly developed. Ovary median, about in mid-body, considerably posterior to testes. Vitelline glands a short distance posterior to ovary, forming a median, rosette-like group, lobes varying in shape typically much broader distally. A living specimen showed that the four-lobed vitelline gland was directly anterior to the three-lobed gland. The usual position of these glands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21

is side by side. The uterus extends to near the posterior end of the body, then coils forward. Eggs 22–25 × 10–12μ. Median stem of the excretory system long, branching a short distance posterior to the ventral sucker. The branches unite dorsal to the oral sucker.

This species is in some respects intermediate between *Hysterolecitha rosea* and *Sterrhurus fusiformis*. It resembles the latter in: testes close together, short genital sinus and prostate, position of genital pore, and relative sizes of suckers; but differs in lacking tail appendage, in position of ovary and vitellaria, and in posterior extent of caeca. It differs from *H. rosea* in lacking oesophagus, in position of genital pore, in more posterior extent of uterus, in sucker ratio, in greater proportion of body posterior to ventral sucker, in shape of vitelline lobes, and in its shorter seminal vesicle. Hysterolecitha differs from the sub-family Sterrhurinae in lacking a tail appendage, but the strong resemblances of the above species to *St. fusiformis* warrants its position in this group.

Sub-family LECITHASTERINAE.

23. **Lecithaster gibbosus** (Rud.) (Figs. 22 and 23).

Host: *Menidia menidia*, silversides.
Position: intestine. Frequency: common.

It was at first intended to consider this form a new species. It seems to differ from *L. gibbosus* in more elongate lobes of ovary; the broad, widely spread vitelline lobes; the far anterior position of the testes; and the more elongate body shape. Variability in these features, however, should be more definitely known.

24. **Brachadena pyriformis** Linton 1910.

Synonyms: *Distomum bothryophoron* Olsson of Linton 1905, pp. 378, 397; *Lecithaster anisotrema* MacCallum 1921.
Hosts: *Micropogon undulatus*, croaker; *Orthopristis chrysopterus*, hogfish.
Position: intestine. Frequency: not very common.

The chief difference between *Brachadena* and *Lecithaster* is in the posterior extent of the seminal vesicle. In this species, the size of the ventral sucker varies from about twice to three times the size of the oral sucker. Body shape is also rather variable. *Lecithaster anisotrema* MacCallum (1921, p. 183) must be considered a synonym of *Brachadena pyriformis*.

Figures 17–25. For lettering see p. 399.

Fig. 17. *Sterrhurus monticellii* from *Pomatomus saltatrix*, ventral view.
Fig. 18. *Lecithochirium synodi* from *Synodus foetens*, ventral view.
Fig. 19. *L. synodi*, portion of sagittal section.
Fig. 20. *L. synodi*, enlarged portion of ventral surface.
Fig. 21. *Hysterolecitha elongata* from *Mugil cephalus*, ventral view.
Fig. 22. *Lecithaster gibbosus* (?) from *Menidia menidia*, ventral view.
Fig. 23. *L. gibbosus*, ventral view of anterior portion.
Fig. 24. *Dinurus magnus* from *Synodus foetens*, ventral view.
Fig. 25. *Dinurus magnus*, ventral view of anterior portion.

Sub-family DINURINAE.

25. **Dinurus magnus** n.sp. (Figs. 24 and 25).

Synonym: *Dist. tornatum* of Linton 1905, e.p.

Hosts: *Synodus foetens*, lizard fish; *Cynoscion nebulosus*, spotted trout.

Position: stomach. Frequency: two in one of two lizard fishes. Several specimens from Cynoscion were immature and occurred in the stomach and air bladder.

Body elongate, very muscular, 11–22 × 1·75–2·27 mm. The tail appendage about two-thirds total length. One of the immature forms from *Cynoscion* was 6·5 mm. in length. This large size is also attained by *Dinurus rubeus* which may become 25 mm. in length. The ventral sucker is slightly more than twice the size of the oral sucker. The genital pore is at the level of the posterior edge of the pharynx. Pre-pharynx absent, oesophagus present, caeca reaching to the end of the tail. Testes oblique, close together, a short distance posterior to the ventral sucker. The seminal vesicle is large, not clearly divided into three parts, extending between the two testes. The pars prostatica is an exceedingly long, coiled tube, extending from the seminal vesicle to the sinus sac. Its coils extend appreciably both posterior and anterior to the ventral sucker. It becomes a straight tube with very few gland cells in that portion dorsal to the ventral sucker. Even in the immature specimens, it extends appreciably posterior and anterior to the ventral sucker. The sinus sac is muscular, cylindrical, about 0·14 mm. in length in the 11 mm. specimen. There is a genital atrium about one-third as long as the sinus sac. Ovary bean-shaped, median, a short distance posterior to the testes; seminal receptacle, large, posterior to ovary. Vitelline glands lateral, consisting of coiled tubes (four on left, three on right). Uterus coils posteriorly about half the length of the long tail, then anteriorly to the sinus sac. Eggs 9–11 × 6–8μ.

This species is very similar to *D. rubeus* in which, however, the pars prostatica is limited to the region dorsal to the ventral sucker. The intrusion of the seminal vesicle between the testes in *D. magnus* may be another less significant difference.

UNCLASSIFIED.

26. **Pleorchis polyorchis** (Stoss.).

Hosts: *Cynoscion regalis*, grey trout; *Cynoscion nebulosus*, spotted trout.

Frequency: in one of six grey trout; in one of six spotted trout.

27. **Siphodera vinaledwardsii** (Linton) Linton.

Synonym: *Monostomum vinaledwardsii* Linton 1901.

Hosts: *Opsanus tau*, toadfish; *Sciaenops ocellatus*, red drum.

Position: intestine. Frequency: common in toadfish; one specimen from one red drum (new host).

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NOTE ON THE OCCURRENCE OF SARCOCYSTIS IN MUSCLE OF PYTHON.

By O. W. TIEGS.

From the Department of Zoology, University of Melbourne.

(With 4 Figures in the Text.)

Although Sarcocystis infections are very common in the muscle of mammals, and to a less extent of birds, in reptiles they have only rarely been seen. In a list given by Wenyon (1926) only two forms are recorded, namely from the muscles of African geckoes and of the lizard *Gongylus*. The form here described was found in fairly large numbers in the striated muscle tissue of the Australian carpet snake *Python spilotes*. For the parasite the name *Sarcocystis pythonis* is suggested.

In size the cysts vary greatly. The largest measured about 1·1 mm. in length. They possess the typical cylindrical form, with rounded and tapering ends. All the forms that I have seen are situated within the muscle fibres, and nothing corresponding to the relatively large adult cysts of *S. tenella* has been found.

Observation of the structure of the contents of large cysts is rendered difficult by the closeness with which the cells are packed. In thin sections two types of cell are recognisable. These are rounded or irregularly polygonal cells, evidently the sporoblasts, and have an appearance similar to those figured by Hartmann and Schilling (1917) from a very young tenella cyst (Fig. 3). But scattered among these are the spores. Some cysts may contain spores only.

The latter have the typical crescent shape. They are only about 4–7μ in length, and are therefore smaller than the well-known tenella spores. The nucleus lies sometimes in the mid-region, sometimes to one end. A minority of spores shows, at one end, a large "vesicle" as described in some other species. Whether this represents a polar capsule with unextruded thread, as van Eecke and Rh. Erdmann (1910) believe, remains undetermined.

In larger specimens the cyst wall is usually distinct, though very thin (much thinner than that commonly seen in *S. tenella*), and I have never seen the striations which are often so obvious in the latter. Sometimes from the membrane transverse septa may develop, dividing the cyst into several successive compartments (Fig. 1), but in the majority of cysts this does not occur.

Associated with the cyst wall are large nuclei. They lie outside the wall within a finely granular, scarcely visible cytoplasm (Fig. 1). They are somewhat shorter and thicker than the nuclei of the muscle tissue, and lack their

characteristic longitudinal polarity. They are probably not muscle nuclei, therefore this argues in favour of Chatton and Avel's view (1923) that the cyst wall is derived from the parasite and not from the muscle fibre, but the evidence is inconclusive.

Some of the large cysts are in advanced stages of degeneration. In some of them scattered spores are still present within a degenerated mass of debris. Sometimes spores seem to be absent, and the interior of the cyst may then present the appearance of an extremely thin-walled recticulum, similar to that

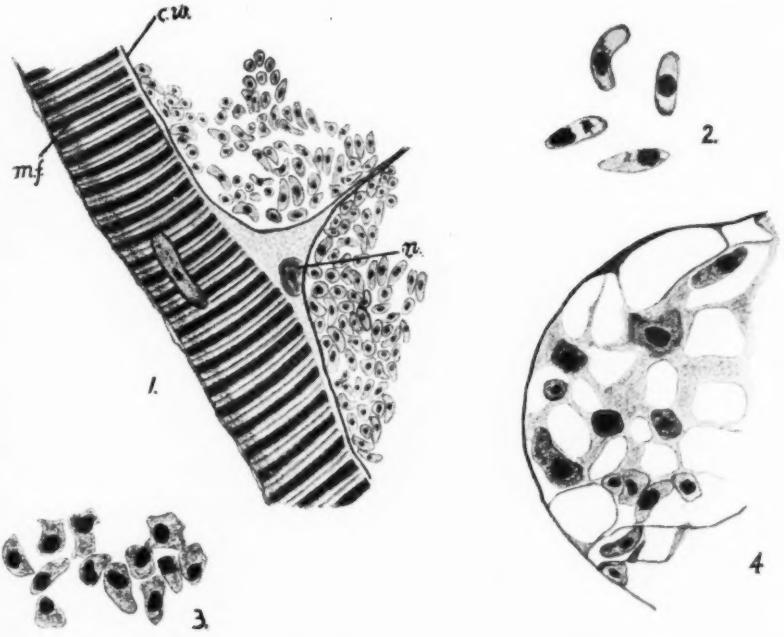

Fig. 1. Section of the outer margin of the cyst, showing formation of transverse septum. *m.f.* part of parasitised muscle fibre; *c.w.* cyst wall; *n.* a nucleus associated with the cyst wall, and seen in optical transverse section. Zeiss apochromat. 2 mm. oc. × 10.

Fig. 2. Four spores. In only one is a definite vesicle seen at one pole. Zeiss apochromat. 2 mm. oc. × 18.

Fig. 3. Mass of cells, evidently mainly sporoblasts, from a large cyst. Zeiss apochromat. 2 mm. oc. × 18.

Fig. 4. Thin section taken at end of a large cyst to show reticular formation of the intercellular substance. Numerous sporoblasts and several spores present.

All specimens drawn from formalin fixed, haematoxylin stained sections.

found in the deeper portions of very large *tenella* cysts, but less distinct and of a much finer texture. The "chambers" are filled with masses of debris. This very fine reticulum is sometimes, though with difficulty, observable in normal

cysts, especially at their margins, and evidently offers a support for the contained spores and sporoblasts (Fig. 4).

Although the parasite completely destroys that part of the muscle fibre which it occupies, it seems to have no deleterious effect on the remainder of the fibre. For example, in Fig. 1 the portion of the muscle fibre immediately adjacent to the parasite does not show any visible abnormality.

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